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- Estrogen receptor
- The present invention relates to isolated DNA (21) The present invention relates to isolated DNA encoding novel estrogen receptors, the proteins encodenceding novel estrogen receptors. (54)

ed by said DNA, chimeric receptors comprising parts of said novel receptors and uses thereof.

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Description

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This invention relates to the field of receptors belonging to the superfamily of nuclear hormone receptors, in parties invention relates to the field of receptors belonging to the superfamily of nuclear hormone receptors, in parties invention relates to DNA annothing a novel eternic recentor the organization of each to the field of receptors belonging to the superfamily of nuclear hormone receptors, in parties invention relates to DNA annothing a novel eternic recentor.

This invention relates to the field of receptors belonging to the superlatinity of nuclear normone receptors. In particular to steroid receptor. The invention relates to DNA encoding a novel steroid receptor. The preparation of said receptor the recentor protein, and the uses thereof epior. The receptor plotein, and the uses thereon.

Steroid hormone receptors belong to a supertamily of nuclear hormone receptors involved in ligand-dependent particles and receptors belong to a supertamily of nuclear hormone receptors belong to a supertamily consists of recentors for non-staroid hormone appropriate the supertamily consists of recentors for non-staroid hormone.

Transcriptional control of gene expression. In addition, this superfamily consists of receptors for non-steroid hormones transcriptional control of gene expression. In addition, this superfamily consists of receptors for non-steroid hormones and relinoids (Gionare et al. Nature 330, 624-629, 1987; Evans, R.M. Science suitamine D. Invroid hormones and relinoids (Gionare et al. Nature 330, 624-629, 1987; Evans, R.M. Science transcriptional control of gene expression. In addition, this superfamily consists of receptors for non-steroid normones and retinoids (Giguère et al, Nature 330, 624-629, 1987; Evans, R.M., Science such as vitamine D, thyroid hormones and retinoids (Giguère et al, Nature 340, 544-649), 1988). Moreover, a range of nuclear recentor-like segmences have been identified which encode social and the segmences have been identified which encode social and the segmences have been identified which encode social and the segmences have been identified which encode social and the segmences have been identified which encode social as the segmences have been identified which encode social and th receptor, the receptor protein, and the uses thereof. such as vitamine U, inyroid normones and retinoids (Giguere et al, Nature 330, 524-529, 1967. Evans, n.iv., Science 240, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encode socalled 240, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 240, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encodes socalled 250, 389-895, 1988). 240. 389-895, 1988). Moreover, a range of nuclear receptor-like sequences have been identified which encode socialed forphan' receptors: these receptors are structurally related to and therefore classified as nuclear receptors. In Manager 126, 1119-1170, 1989. D. I. Manager 1989 for number of numbers of the properties of the propertie orphan receptors: these receptors are structurally related to and therefore classified as flucted receptors, authough no putative ligands have been identified yet (B.W. O'Malley, Endocrinology 125, 1119-1170, 1989; D.J. Mangelsdorf and R.M. Evans Cell. 83, 841-850, 1995)

I.N.N. Evens, Cell, 22, 241-220, 1993).

The superfamily of nuclear hormone receptors share a modular structure in which six distinct structural and functions that the superfamily of nuclear hormone receptors share a modular structure in which six distinct structural and functions.

The superfamily of nuclear hormone receptors share a modular structure in which six distinct structural and functions.

The superfamily of nuclear hormone receptors share a modular structure in which six distinct structural and functions.

The superramily of nuclear normone receptors share a modular structure in which six distinct structural and functional domains, A to F, are displayed (Evans, Science 240, 889-895, 1988). A nuclear hormone receptor is characterized tional domains, A to F, are displayed (Evans, Science 240, 889-895, 1988). A nuclear hormone receptor is characterized tional domains, A to F, are displayed (Evans, Science 240, 889-895, 1988). A nuclear hormone receptor is characterized to the follower have contrally located in highly conserved DNA-hindring domain A/R). Tollower have centrally located in hindly conserved DNA-hindring have a variable N-terminal region (domain A/R). tional domains. A to h, are displayed (Evans, Science 240, 889-895, 1988). A nuclear hormone receptor is characterized by a variable N-terminal region (domain A/B), followed by a centrally located, highly conserved DNA-binding domain by a variable N-terminal region (domain A/B), a variable hinder region (domain D), a conserver linand-hinding domain (hereinafter referred to as DRD; domain C), a variable hinder region (domain D) a conserver linand-hinding domain D). and R.M. Evans, Cell, <u>83</u>, 841-850, 1995).

by a variable in-terminal region (domain PD), rollowed by a certifally located, myriny conserved branch domain of the related to as DBD; domain C), a variable hinge region (domain D), a conserved ligand-binding domain therein after referred to as DBD; domain E) and a variable C-terminal region (domain E). telli after referred to as LDU, domain E) and a variable U-terminal region (domain F).

The N-terminal region, which is highly variable in size and sequence, is poorly conserved among the different into the modulation of transcription activation (Rocque). The N-terminal region, which is highly variable in size and sequence, is poorly conserved among the different into the modulation of transcription activation (Rocque). (herein after referred to as LBD; domain E) and a variable C-terminal region (domain F).

The IN-Terminal region, which is highly variable in size and sequence, is poonly conserved among the different members of the superfamily. This pan of the receptor is involved in the modulation of transcription activation (Bocquel members of the superfamily. This pan of the receptor is involved in the modulation of transcription activation (Bocquel members of the superfamily. This pan of the receptor is involved in the modulation of transcription activation (Bocquel Machine Machine).

II, NUCL. ACID MES., 17, 2001-2000, 1969; Tora et al., Cell 59, 477-467, 1989).

The DBD consists of approximately 66 to 70 amino acids and is responsible for DNA-binding activity: it targets the approximately 66 to 70 amino acids and is responsible for DNA-binding activity: it targets the approximately for the specific DNA sequences called hormone responsive elements (hereinafter reterred to as LIDE) within the approximately for the specific DNA sequences called hormone responsive elements. receptor to specific DNA sequences called hormone responsive elements (hereinafter referred to as HRE) within the et al, Nucl. Acid Res., 17, 2581-2595, 1989; Tota et al, Cell 59, 477-487, 1989).

receptor to specific UNA sequences called normone responsive elements (hereinatter reterred to as HHE) within the transcription control unit of specific target genes on the chromatin (Martinez and Wahli, In 'Nuclear Hormone Receptors', Acad. Press. 125-153. 1991) 10. Press, 123-153, 1331).

The LBD is located in the C-terminal part of the receptor and is primarily responsible for ligand binding activity. In the LBD is located in the C-terminal part of the hormone linand and in addition no see each a transcrint line of the hormone linand and in addition no see each at the land in addition of the hormone linand and in addition no see each at the land is decential for recognition and hinding of the hormone linand and in addition no see each at the land is decential for recognition and hinding of the hormone linand and in addition no see each at the land is primarily responsible for ligand binding activity. In the land is primarily responsible for ligand binding activity.

this way, the LBD is essential for recognition and binding of the hormone ligand and, in addition possesses a transcription activation function thereby determining the specificity and selectivity of the hormone response of the recents ITHS WAY, THE LED IS ESSENTIAL FOR RECOgnITION and Dinding OF the normone ligand and, in addition possesses a transcription activation function, thereby determining the specificity and selectivity of the hormone response of the receptor. It is activation function, thereby determining the PRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology heliwent the LRO's are known to vary considerably in hormology. Iton activation function, inereby determining the specificity and selectivity of the normone response of the author between the indi-Although moderately conserved in structure, the LBD's are known to vary considerably in homology between the indi-windust members of the author between reconstruction and authorized property of the author between the authorized property of the author between reconstructions and authorized the authorized property of t Although moderately conserved in structure, the LBU's are known to vary considerably in homology between the individual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, FASEB vidual members of the nuclear hormone receptor superfamily (Evans, Science 240, 889-895, 1988; P.J. Fuller, F Acad. Press, 125-153, 1991). 25

2, JUNE JUNES, 1991, Mangelsuon et al. Cell, vol. 83, 835-839, 1995).

Functions present in the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other and it has been according from the N-terminal region, LBD and DBD operate independently from each other according from the N-terminal region, LBD and DBD operate independently from each other according from the N-terminal region, LBD and DBD operate independently from each other according from the N-terminal region according from the N-terminal FUNCTIONS present in the N-terminal region, LBD and DBD operate independently from each other and it has been shown that these domains can be exchanged between nuclear receptors (Green et al., Nature, Vol. 325, 75-78, 1987).

This results in chimeric nuclear recentors, such as described for instance in WO-4-AGORGES. Vidual members of the nuclear normalie receptor superiority (CVaris, out).

J. 5, 3092-3099, 1991; Mangelsdorf et al. Cell, Vol. 83, 835-839, 1995). 30

Stesulis in chimetic nuclear receptors, such as described for instance in WO-A-BUDGED. By the LBD, it will bind.

When a hormone ligand for a nuclear receptor enters the cell by diffusion and is recognized by the LBD, it will bind.

When a hormone ligand for a nuclear receptor enters the cell by diffusion and is recognized. As a result of this he specific recentor protein, thereby initiating an alloctaric alteration of the recentor protein. Shown that these domains can be exchanged between nuclear receptors (Green et al., Nature).

This results in chimeric nuclear receptors, such as described for instance in WO-A-8905355.

Writen a normone liganution a nuclear receptor enters the cell by diffusion and is recognized by the LDU, it will bind to the specific receptor protein, thereby initiating an allosteric alteration of the receptor protein, hereby initiating an allosteric alteration of the receptor protein, thereby initiating an allosteric alteration of the receptor protein, thereby initiating an allosteric alteration and as such is able to bind through the specific receptor protein, thereby initiating an allosteric alteration of the receptor protein. to the specific receptor protein, intereby initiating an allosteric alteration of the receptor protein. As a result of this able to bind through alteration the ligand/receptor complex switches to a transcriptionally active state and as such is able to bind through alteration the ligand/receptor complex switches to a transcriptionally active state and as such is able to bind through the research of the DRD with high affinity to the corresponding HRF on the chromatin DNA (Martinez and Wahl). atteration the ligand/receptor complex switches to a transcriptionally active state and as such is able to bind through the presence of the DBD with high affinity to the corresponding HRE on the chromatin DNA (Martinez and Wahli, the presence of the DBD with high affinity to the corresponding the linany/recentor complex modulates extinuously th the presence of the UBU with high affinity to the corresponding HHL on the chromatin UNA (Martinez and Wahli, which is the corresponding HHL on the chromatin UNA (Martinez and Wahli, and presence of the UBU with high affinity to the corresponding HHL on the chromatin UNA (Martinez and Wahli, which is the corresponding HHL on the chromatin UNA (Martinez and Wahli, and the corresponding HHL on the chromatin UNA (Martinez and Wahli, which is the corresponding HHL on the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high and the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high affinition of the chromatin UNA (Martinez and Wahli, and the chromatin UNA) with high and the chromatin UNA (Martinez and Wahli, and the chr Nuclear Hormone Receptors, 120-103, Acad. Press, 1991). In this way the liganomeceptor complex modulates expression of the specific target genes. The diversity achieved by this family of receptors results from their ability to respond to different ligands.

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pond to different ligarias.

The steroid hormone receptors are a distinct class of the nuclear receptor superfamily, characterized in that the andro-Ine steroid normone receptors are a distinct class of the nuclear receptor superfaiting, characterized in that the ligands are steroid hormones. The receptors for glucocorticoids (GR), mineralcorticoids (MR), progestins (PR), and or ligands are steroid hormones. The receptors for glucocorticoids (GR), mineralcorticoids (GR), mineralcorticoids (MR), progestins that the unique liganus are sieroid normones. The receptors for glucocorricolus (UR), mineralcorricolus (WR), progestins (PR), ando-gens (AR) and estrogens (ER) are classical steroid receptors. Furthermore, the steroid receptors The CR MR DR

gens (AH) and estrogens (EH) are classical steroid receptors. Furthermore, the steroid receptors have the unique ability upon activation to bind to palindromic DNA sequences, the so-called HRE's, as homodimers. The GR, MR, PR ability upon activation to bind to palindromic DNA sequences, the so-called HRE's, as homodimers. The GR, MR, PR ability upon activation to bind to palindromic DNA sequences, the so-called HRE's, as homodimers. The GR, MR, PR ability upon activation to bind to palindromic DNA sequences, the so-called HRE's, as homodimers. ability upon activation to bind to patindromic DNA sequences, the so-called HHE's, as nomodimers. The GH, MH, PH and AR recognize the same DNA sequence. While the ER recognizes a different DNA sequence. (Beato et al., Cell, and AR recognize the same DNA sequence, while the ER recognizes a different DNA sequence of the head of the same DNA sequence. While the etamoid recentor is thought to interest with components of the head of the same DNA sequence. respond to different ligands. and AH recognize the same DNA sequence, while the EH recognizes a dillerent DNA sequence. (Deato et al., Cell, Vol. 83, 851-857, 1995). After binding to DNA, the steroid receptor is thought to interact with components of the basal vol. 83, 851-857, 1995). After binding to DNA, the steroid receptor is thought to interact with components of shading the expression of shading the exp VUI. 63, 631-637, 1883). After unrung to LIVA, the steroid receptor is mought to interact with components of the basal transcriptional machinery and with sequence-specific transcription factors, thus modulating the expression of specific transcription factors.

get genes.

Several HRE's have been identified, which are responsive to the hormone/receptor complex. These HRE's are several HRE's have been identified, which are responsive to the hormone/receptor complex. These HRE's are several HRE's have been identified, which are responsive to the hormone/receptor complex. These HRE's are several HRE's have been identified, which are responsive to the hormone/receptor complex. These HRE's are several HRE's have been identified, which are responsive to the hormone/receptor complex.

Several RME's have been liderfulled, which are responsive to the normalizate professional control units of the various target genes such as mammalian growth hormone genes situated in the transcriptional control units of the various target genes and professional control units Situated in the transcriptional control units of the various target genes such as mammalian growth normone genes (responsive to glucocorticoid, estrogen, testosterone), mammalian prolactin genes and progesterone receptor genes (responsive to glucocorticoid, estrogen, testosterone), mammalian prolactin genes and progesterone receptor genes (responsive to procesterone), mammalian metallothionein genes (responsive to procesterone). (responsive to glucocorticold, estrogen, testosterone), mammalian protectin genes and progesterone receptor genes (responsive to progesterone), mammalian metallothionein gene (responsive to Estrogen), avian ovalbumin genes (responsive to progesterone), mammalian heatic α . -alabulin gene (responsive to estrogen) to strong to allicocorticold) and mammalian heatic α . -alabulin gene (responsive to estrogen). (responsive to Estrogen), avian ovalbumin genes (responsive to progesterone), mammalian metallothionein gene (responsive to Estrogen), avian ovalbumin genes (responsive to progesterone), mammalian metallothionein gene (responsive to cstrogen, testosterone, glucosponsive to glucocorticoid) and mammalian hepatic $\omega_{2\mu}$ -globulin gene (responsive to cstrogen, testosterone, glucocorticoid). icold).

The steroid hormone receptors have been known to be involved in embryonic development, adult homeostasis as

The steroid hormone receptors have been known to be involved in embryonic development, adult homeostasis as

The steroid normone receptors have been known to be involved in embryonic development, adult nomeostasts as well as organ physiology. Various diseases and abnormalities have been ascribed to a disturbance in the steroid moderate pathway. Since the steroid recentors everying their influence as hormone adjusted transactional modulation. well as organ physiology. Various diseases and abnormalities have been ascribed to a disturbance in the steroid hormone pathway. Since the steroid receptors exercise their influence as hormone-activated transcriptional modulators, mone pathway. Since the steroid receptors exercise their influence as well as overstimulation or blocking of these in these recentors as well as overstimulation or blocking of these in these recentors. mone painway. Since the steroic receptors exercise their influence as normone-activated transcriptional modulators, it can be anticipated that mutations and defects in these receptors, as well as overstimulation or blocking of these it can be anticipated that mutations and defects in these receptors. receptors might be the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanism of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanism of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanism of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanisms of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanisms of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanisms of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanisms of the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanisms of the underlying reason for the altered pattern. A better knowledge of these receptors is the underlying reason for the altered pattern. A better knowledge of these receptors is the underlying reason for the underlying rea receptors might be the underlying reason for the altered pattern. A better knowledge of these receptors, their mechanism of action and of the ligands which bind to said receptor might help to create a better insight in the underlying reason for the disparse which eventually will lead to hetter treatment of the disparse mechanism of the hormone sinnal transduction nathway which eventually will lead to hetter treatment of the hormone sinnal transduction nathway. nism of action and of the ligands which bind to said receptor might help to create a better insight in the underlying mechanism of the hormone signal transduction pathway, which eventually will lead to better treatment of the diseases mechanism of the hormone signal transduction pathway.

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abnormalities linked to altered normone/receptor functioning.

For this reason cDNA's of the steroid and several other nuclear receptors of several mammalians, including huse for this reason cDNA's of the steroid and several other nuclear receptors of several mammalians, including huse for this reason cDNA's of the steroid and several other nuclear receptors of several mammalians, including huse for this reason cDNA's of the steroid and several other nuclear receptors of several mammalians, including huse for this reason cDNA's of the steroid and several other nuclear receptors of several mammalians. and abnormalities linked to altered hormone/receptor functioning.

mans, have been isolated and the corresponding amino acid sequences have been deduced, such as for example the human steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors for vitamine D, thyroid hormones, human steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors PR, ER, GR, MR, and AR, the human non-steroid receptors for vitamine D, thyroid hormones, and retinoid acid. In addition, children acid in addition, children acid and retinoid acid. In addition, children acid and retinoid acid. human steroid receptors PH, EH, GH, MH, and AH, the human non-steroid receptors for vitamine D, thyroid hormones, and retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan read retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinoid a retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinoid a retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinoid a retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids such as retinoid a retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan retinoids acid. and retinoids such as retinol A and retinoic acid. In addition, cDNA's encoding well over 100 mammalian orphan receptors have been isolated, for which no putative ligands are known yet (Mangelsdorf et al. Cell, Vol.83, 835-839,
ceptors have been isolated, for which no putative ligands are known yet (mangels in order to unrave) the various ceptors have been isolated, for which no putative ligands are known yet (Mangelsdori et al. Cell, Vol.33, 833-839, 1995). However, there is still a great need for the elucidation of other nuclear receptors in order to unravel the various roles these recentors play in normal physiology and nathology.

is these receptors play in normal physiology and pathology.

The present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. The present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. More specific, the present invention provides for such a novel nuclear receptor. Said novel steroid receptors are novel estroid receptors. In the present invention provides for such a novel nuclear receptor. Said novel steroid receptors are novel estroid receptors are novel estroid receptors. The nuclear receptor is not a novel nuclear receptor. The nuclear receptor is not such as a novel nuclear receptor is not such as a novel nuclear receptor. The nuclear receptor is not such as a novel nuclear receptor is not such as a n roles these receptors play in normal physiology and pathology.

which are able to bind and be activated by, for example, estradiol, estrone and estriol.

According to the present invention it has been found that a novel estrogen receptor is expressed as an 8 kb.

According to the present invention if has been found that a novel estrogen receptor.

According to the present invented paraboral blood timeshouter (pare) over and tention and the present in human thirdle estable. tor novel steroid receptors, having estrogen mediated activity. Said novel steroid receptors and estroid tors, which are able to bind and be activated by, for example, estradiol, estrone and estroid tors, which are able to bind and be activated if her hard found that a could estrone recent.

According to the present invention it has been found that a novel estrogen receptor is expressed as an & kb transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. Furthermore, and shleen transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. Furthermore, additional transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. Furthermore, additional transcript in human thymus, spleen, peripheral blood lymphocytes (PBLs), ovary and testis. Furthermore, additional transcript and spleen.

These transcripts are prohably generally alternative spleen. transcripts have been identified. Another transcript of approximately 10 kb was identified in ovary, thymus and spiech.

Intestis, an additional transcript of 1.3 kb was detected. These transcripts are probably generated by alternative splicing of the open anothing the povel estronger recentor according to the invention.

ne gene encoding the novel estrogen receptor according to the invention.

Cloning of the cDNA's encoding the novel estrogen receptors according to the invention revealed that several coloring of the cDNA's encoding the novel estrogen receptors according to the invention revealed that several coloring to the cDNA's encoding the novel estrogen receptors according to the invention revealed that several coloring to the coloring to Cloning of the cDNA's encoding the novel estrogen receptors according to the invention revealed that several splicing variants of said receptor can be distinguished. At the protein level, these variants differ only at the C-terminal splicing variants of said receptor can be distinguished. of the gene encoding the novel estrogen receptor according to the invention. 20

CDNA encoding an ER has been isolated (Green, et al, Nature 320, 134-139, 1986; Greene et al, Science 231, or control of the recent of the rec part.

cunal encoding an EH has been isolated (Green, et al., Nature 320, 134-139, 1986; Greene et al., Science 231, 1150-1154, 1986), and the corresponding amino acid sequence has been deduced. This receptor and the receptor acid invention however are distinct, and encoded for his different genes with different nucleic acid according to the present invention however are distinct. 1150-1154, 1986), and the corresponding amino acid sequence has been deduced. This receptor and the receptor according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid according to the present invention. according to the present invention, however, are distinct, and encoded for by different genes with different nucleic acid the ER according to the ER according to the sequences. Not only do the ER of the prior art (hereinafter referred to as classical ER) and the ER according to the sequences. Not only do the ER of the prior art (hereinafter referred to as classical ER) and the ER according to the sequences. sequences. Not only do the ER of the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the classical ER is located on the prior art (hereinafter referred to as classical ER) and the ER according to the invention was found the prior art (hereinafter referred to as classical ER) and the ER according to the invention was found the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the ER according to the prior art (hereinafter referred to as classical ER) and the ER according to the ER accordi present invention diller in amino acid sequence, they also are located on different chromosomes. The gene encouning the ER according to the invention was found the classical ER is located on chromosome 6, whereas the gene encoding the ER according to the invention furthermore distinguishes itself from the classical to the located on chromosome 14. The ER according to the invention furthermore distinguishes itself from the classical to the located on chromosome 14. the classical ER is located on chromosome 6, whereas the gene encoding the ER according to the invention was found to be located on chromosome 14. The ER according to the invention furthermore distinguishes itself from the classical to be located on chromosome 14. The ER according to the invention furthermore helwen these recentors in differences in tiesual distribution. to be located on chromosome 14. The EH according to the invention turthermore distinguishes lisely from the classical receptor in differences in tissue distribution, indicating that there may be important differences between these receptors at the level of detropodic simpalling.

ne level of estrogenic signalling.
In addition, two orphan receptors, ERRα and ERRβ, having an estrogen receptor related structure have been in addition, two orphan receptors, entired to have a contract to have been a contract to have a contract to have been a contract to have a contract to have been a contract to have a contract to hav In addition, two orphan receptors, EHH α and EHH β , having an estrogen receptor related structure have been described (Giguere et al., Nature 331, 91-94, 1988). These orphan receptors, however, have not been reported to be described (Giguere et al., Nature 331, 91-94, 1988). These orphan receptors, however, have not been reported to the classical ER and other linands which hind to the classical ER and other linands which hind to the classical ER. at the level of estrogenic signalling.

described (Giguère et al., Nature 331, 91-94, 1988). These orphan receptors, however, have not been reported to be these orphan receptors, however, have not been reported to these orphan receptors, however, have not been reported to these orphan receptors, however, have not been reported to these orphan receptors and other ligands which bind to these orphan receptors have not been found vet. The novel estronen recentor according to the invention distinguishes itself clearly receptors have not been found vet. able to bind estrodial or any other hormone that binds to the classical EH, and other ligands which bind to these receptors have not been found yet. The novel estrogen receptor according to the invention distinguishes itself clearly receptors have not been found yet. The novel estrogen receptor according to the invention distinguishes itself clearly receptors have not been found yet. The novel estrogen receptor according to the invention distinguishes itself clearly receptors have not been found to bind estrogens. IT ITIES E TECEPIOLS SITURE IT WAS TOUTHU TO DITU ESTROGERS.

THE fact that a novel ER according to the invention has been found is all the more surprising, since any suggestion of the fact that a novel ER according to the invention was absent in the scientific literature, neither the isolation of was absent in the scientific literature.

The fact that a novel EH according to the invention has been found is all the more surprising, since any suggestion towards the existence of additional estrogen receptors was absent in the scientific literature: neither the isolation of the cristence of additional estrogen receptors was absent in the scientific literature: neither the oresence of additional estrogen receptors was absent in the scientific literature: neither the oresence of additional estrogen receptors was absent in the scientific literature: neither the oresence of additional estrogen receptors. towards the existence of additional estrogen receptors was absent in the scientific literature; neither the isolation of the classical ER nor the orphan receptors ERRic and ERRip suggested or hinted towards the presence of additional the classical ER nor the orphan receptors ERRic and ERRip suggested or hinted towards the presence of additional ER's could be a classical ER nor the orphan receptors according to the invention. The identification of additional ER's could be a controlled to the invention. from these receptors since it was found to bind estrogens. the classical EH nor the orphan receptors EHH α and EHH β suggested or hinted towards the presence of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptors such as the receptors according to the invention. The identification of additional ER's could be a estrogen receptor according to the invention. estrogen receptors such as the receptors according to the invention. The identification of additional Ensignment be assuch ascribe major step forward for the existing clinical therapies, which are based on the existence of one ER and as such ascribe major step forward for the existing clinical therapies, which are based on the existence according to the invention will all setting mediated abnormalities and/or dispasse to this one recentor. The recentors according to the invention will be tropped mediated abnormalities and/or dispasse to this one recentor. major step forward for the existing clinical therapies, which are based on the existence of one ER and as such ascribe all estrogen mediated abnormalities and/or diseases to this one receptor. The receptors according to the invention will estrogen mediated abnormalities and/or diseases to this one receptor. The receptors according to the novel estronen and estrogen mediated abnormalities and/or diseases to this one receptor. all estrogen mediated approximations and/or diseases to this one receptor. The receptors according to the invention will be useful in the development of hormone analogs that selectively activate either the classical ER or the novel estrogen be useful in the development of hormone analogs that selectively activate either the classical ER or the novel estrogen be useful in the development of hormone analogs that selectively activate either the classical ER or the novel estrogen be useful in the development of hormone analogs that selectively activate either the classical ER or the novel estrogen because of the novel estrogen according to the invention. This should be considered as one of the major advantages of the nessent invention. be useful in the development of normone analogs that selectively activate either the classical EM or the novel estroyers receptor according to the invention. This should be considered as one of the major advantages of the present invention. This is one aspect, the present invention provides for isolated CDNA encoding a novel steroid recentor. In particular, the present invention provides for isolated CDNA encoding a novel steroid recentor. splor according to the invention. This should be considered as one of the major advantages of the present invention.

Thus, in one aspect, the present invention provides for isolated cDNA encoding a novel serior recentor.

lar, the present invention provides for isolated cunk encoding a novel estrogen receptor.

According to this aspect of the present invention, there is provided an isolated DNA encoding a steroid receptor and a transfer in the amino acid the present invention, there is provided an isolated DNA encoding the amino acid according to this aspect of the present invention, there is provided an isolated DNA encoding the amino acid the present invention domain and a transfer invention domain. ticular, the present invention provides for isolated cDNA encoding a novel estrogen receptor.

According to this aspect of the present invention, there is provided an isolated DNA encoding a steroid receptor protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid protein having an N-terminal domain, a DNA-binding domain and a ligand-binding homology with the amino acid sequence of said DNA-binding domain of said recently orders exhibits at least any homology with the amino acid protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 80% homology with the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 80% homology with the amino acid sequence of said linand-binding domain of said recentor notein sequence of said DNA-binding domain of said recentor notein sequence shown in SFQ ID NO 3 and the amino acid sequence of said linand-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 80% homology with the amino acid sequence of said ligand-binding domain of said receptor protein sequence shown in SEQ ID NO:3, and the amino acid sequence shown in SEQ ID NO:4

Sequence shown in SEQ ID NO:4

nibits at least 70% nomology with the amino acid sequence shown in SEQ 10 NO.4.

In particular, the isolated DNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding In particular, the isolated DNA encodes a steroid receptor protein having an NA-binding domain wherein the amino acid sequence of said DNA-binding domain wherein the amino acid sequence of said DNA-binding domain. sequence snown in SEQ ID NO.4. and the amino acid sequence of salo ligand-ornaing of exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO.4. In particular, the isolated UNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding an N-terminal domain, a DNA-binding an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor protein exhibits at least 90%, preferably 95%, more preferably 98%, most preferably 100% homology with the amino acid sequence shown in SEO ID NO:3

d sequence shown in SEC ID NO.3.

More particularly, the isolated DNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding more particularly, the isolated DNA encodes a steroid receptor protein having an N-terminal domain of said recentor wherein the amino anid sequence of said linand-hinding domain wherein the amino anid sequence of said linand-hinding domain wherein the amino anid sequence of said linand-hinding domain.

More particularly, the isolated DNA encodes a steroid receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain of said receptor across a steroid receptor protein having an N-terminal domain of said receptor domain and a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain, a DNA-binding domain of said receptor protein having an N-terminal domain, a DNA-binding domain of said receptor protein having an N-terminal domain of said receptor domain and a ligand-binding domain of said receptor protein having an N-terminal domain of said recepto domain and a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain of said receptor, wherein the amino acid sequence of said ligand-binding domain of said receptor, which is a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain of said receptor, which is a ligand-binding domain of said receptor, which is a ligand-binding domain of said receptor. acid sequence shown in SEQ ID NO.3.

acid sequence shown in SEQ ID NO:4.

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A preferred isolated DNA according to the invention encodes a steroid receptor protein having the amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.

A more preferred isolated DNA according to the invention is an isolated DNA comprising a nucleotide sequence stown in SEC ID NO:1, SEQ ID NO:2, SEQ ID NO:20 or SEQ ID NO:24.

The DNA according to the invention may be obtained from cDNA. Alternatively, the coding sequence might be genomic DNA or prepared using DNA synthesis techniques.

The DNA according to the invention will be very useful for in vivo expression of the novel receptor proteins according to the invention in sufficient quantities and in substantially pure form.

In another aspect of the invention, there is provided for a steroid receptor comprising the amino acid sequence encoded by the above described DNA molecules.

The steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor exhibits at least 80% homology with the amino acid sequence shown in SEQ ID NO:3, and the amino acid sequence of said ligand-binding domain of said receptor exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO:4.

In particular, the steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said receptor exhibits at least 90% preferably 95%, more preferably 98%, most preferably 100% homology with the amino acid sequence shown in SEC ID NO 3.

More particular, the steroid receptor according to the invention has an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said ligand-binding domain of said receptor exhibits at least 75% prefearbly 80%, more preferably 90%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO 4

It will be clear for those skilled in the art that also steroid receptor proteins comprising combined DBD and LBD preferences and DNA encoding such receptors are subject of the invention.

Preferably, the steroid receptor according to the invention comprises an amino acid sequence shown in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.

Also within the scope of the present invention are steroid receptor proteins which comprise variations in the amino acid sequence of the DBD and LBD without loosing their respective DNA-binding or ligand-binding activities. The variations that can occur in those amino acid sequences comprise deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence, said variations resulting in amino acid difference(s) in the overall sequence. It is well known in the art of proteins and peptides that these amino acid differences lead to amino acid sequences that are different from, but still homologous with the native amino acid sequence they have been derived from

Amino acid substitutions that are expected not to essentially alter biological and immunological activities, have been described in for example Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Arg/Lys, Asp/Asn, Ile/Val. Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous polypeptides.

Variations in amino acid sequence of the DBD according to the invention resulting in an amino acid sequence that has at least 80% homology with the sequence of SEQ ID NO:3 will lead to receptors still having sufficient DNA binding activity. Variations in amino acid sequence of the LBD according to the invention resulting in an amino acid sequence that has at least 70% homology with the sequence of SEQ ID NO:4 will lead to receptors still having sufficient ligand history activity.

Homology as defined herein is expressed in percentages, determined via PCGENE. Homology is calculated as the percentage of identical residues in an alignment with the sequence according to the invention. Gaps are allowed to obtain maximum alignment.

Comparing the amino acid sequences of the classical ER and the ER's according to the invention revealed a high degree of similarity within their respective DBD's. The conservation of the P-box (amino acids E-G-X-X-A) which is responsible for the actual interactions of the classical ER with the target DNA element (Zilliacus et al., Mol.Endo. 9, 389, 1995; Glass. End.Rev. 15, 391, 1994), is indicative for a recognition of estrogen responsive elements (ERE's) by the ER's according to the invention. The receptors according to the invention indeed showed ligand-dependent transactivation on ERE-containing reporter constructs. Therefore, the classical ER and the novel ER's according to the invention may have overlapping target gene specificities. This could indicate that in tissues which co-express both respective ER's, these receptors compete for ERE's. The ER's according to the invention may regulate transcription of target genes differently from classical ER regulation or could simply block classical ER functioning by occupying estrogen responsive elements. Alternatively, transcription might be influenced by heterodimerization of the different

receptors.

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Thus, a preferred steroid receptor according to the invention comprises the amino acid sequence E-G-X-X-A within the P box of the DNA binding domain, wherein X stands for any amino acid. Also within the scope of the invention is isolated DNA encoding such a receptor.

Methods to prepare the receptors according to the invention are well known in the art (Sambrook et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, 1989). The most practical approach is to produce these receptors by expression of the DNA encoding the desired protein.

A wide variety of host cell and cloning vehicle combinations may be usefully employed in cloning the nucleic acid sequence coding for the receptor of the invention. For example, useful cloning vehicles may include chromosomal, non-chromosomal and synthetic DNA sequences such as various known bacterial plasmids and wider host range plasmids and vectors derived from combinations of plasmids and phage or virus DNA. Useful hosts may include bacterial hosts, yeasts and other fungi, plant or animal hosts, such as Chinese Hamster Ovary (CHO) cells or monkey cells and other hosts.

Vehicles for use in expression of the ligand-binding domain of the present invention will further comprise control sequences operably linked to the nucleic acid sequence coding for the ligand-binding domain. Such control sequences generally comprise a promoter sequence and sequences which regulate and/or enhance expression levels. Furthermore an origin of replication and/or a dominant selection marker are often present in such vehicles. Of course control and other sequences can vary depending on the host cell selected.

Techniques for transforming or transfecting host cells are quite known in the art (see, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989).

Recombinant expression vectors comprising the DNA of the invention as well as cells transformed with said DNA or said expression vector also form part of the present invention.

In a further aspect of the invention, there is provided for a chimeric receptor protein having an N-terminal domain, a DNA-binding domain, and a ligand-binding domain, characterized in that at least one of the domains originates from a receptor protein according to the invention, and at least one of the other domains of said chimeric protein originates from another receptor protein from the nuclear receptor superfamily, provided that the DNA-binding domain and the ligand-binding domain of said chimeric receptor protein originate from different proteins.

In particular, the chimeric receptor according to the invention comprises the LBD according to the invention, said LBD having an amino acid sequence which exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO:4. In that case the N-terminal domain and DBD should be derived from another nuclear receptor, such as for example PR. In this way a chimeric receptor is constructed which is activated by a ligand of the ER according to the invention and which targets a gene under control of a progesterone responsive element. The chimeric receptors having a LBD according to the invention are useful for the screening of compounds to identify novel ligands or hormone analogs which are able to activate an ER according to the invention.

In addition, chimeric receptors comprising a DBD according to the invention, said DBD having an amino acid sequence exhibiting at least 80% homology with the amino acid sequence shown in SEQ ID NO:3, and a LBD and, optionally, an N-terminal domain derived from another nuclear receptor, can be succesfully used to identify novel ligands or hormone analogs for said nuclear receptors. Such chimeric receptors are especially useful for the identification of the respective ligands of orphan receptors.

Since steroid receptors have three domains with different functions, which are more or less independent, it is possible that all three functional domains have been derived from different members of the steroid receptor superfamily.

Molecules which contain parts having a different origin are called chimeric. Such a chimeric receptor comprising the ligand-binding domain and/or the DNA-binding domain of the invention may be produced by chemical linkage, but most preferably the coupling is accomplished at the DNA level with standard molecular biological methods by fusing the nucleic acid sequences encoding the necessary steroid receptor domains. Hence, DNA encoding the chimeric receptor proteins according to the invention are also subject of the present invention.

Such chimeric proteins can be prepared by transfecting DHA encoding these chimeric receptor proteins to suitable host cells and culturing these cells under suitable conditions.

It is extremely practical if, next to the information for the expression of the steroid receptor, also the host cell is transformed or transfected with a vector which carries the information for a reporter molecule. Such a vector coding for a reporter molecule is characterized by having a promoter sequence containing one or more hormone responsive elements (HRE) functionally linked to an operative reporter gene. Such a HRE is the DNA target of the activated steroid receptor and, as a consequence, it enhances the transcription of the DNA, coding for the reporter molecule. In *in vivo* settings of steroid receptors the reporter molecule comprises the cellular response to the stimulation of the ligand. However, it is possible *in vitro* to combine the ligand-binding domain of a receptor to the DNA binding domain and transcription activating domain of other steroid receptors, thereby enabling the use of other HRE and reporter molecule systems. One such a system is established by a HRE presented in the MMTV-LTR (mouse mammary tumor virus long terminal repeat sequence in connection with a reporter molecule like the firefly luciferase gene or the bacterial gene

for CAT (chloramphenicol transferase). Other HRE's which can be used are the rat oxytocin promotor, the retinoic acid responsive element, the thyroid hormone responsive element, the estrogen responsive element and also synthetic responsive elements have been described (for instance in Fuller, ibid. page 3096). As reporter molecules next to CAT and luciferase β-galactosidase can be used.

Steroid hormone receptors and chimeric receptors according to the present invention can be used for the *in vitro* identification of novel ligands or hormonal analogs. For this purpose binding studies can be performed with cells transformed with DNA according to the invention or an expression vector comprising DNA according to the invention, said cells expressing the steroid receptors or chimeric receptors according to the invention.

The novel steroid hormone receptor and chimeric receptors according to the invention as well as the ligand-binding domain of the invention, can be used in an assay for the identification of functional ligands or hormone analogs for the nuclear receptors.

Thus, the present invention provides for a method for identifying functional ligands for the steroid receptors and chimeric receptors according to the invention, said method comprising the steps of

- a) introducing into a suitable host cell 1) DNA or an expression vector according to the invention, and 2) a suitable reporter gene functionally linked to an operative hormone response element, said HRE being able to be activated by the DNA-binding domain of the receptor protein encoded by said DNA;
 - b) bringing the host cell from step a) into contact with potential ligands which will possibly bind to the ligand-binding domain of the receptor protein encoded by said DNA from step a);
 - c) monitoring the expression of the receptor protein encoded by said reporter gene of step a).

If expression of the reporter gene is induced with respect to basic expression (without ligand), the functional ligand can be considered as an agonist; if expression of the reporter gene remains unchanged or is reduced with respect to basic expression, the functional ligand can be a suitable (partial) antagonist.

For performing such kind of investigations host cells which have been transformed or transfected with both a vector encoding a functional steroid receptor and a vector having the information for a hormone responsive element and a connected reporter molecule are cultured in a suitable medium. After addition of a suitable ligand, which will activate the receptor the production of the reporter molecule will be enhanced, which production simply can be determined by assays having a sensitivity for the reporter molecule. See for instance WO-A-8803168. Assays with known steroid receptors have been described (for instance S. Tsai et al., Cell 57, 443, 1989; M. Meyer et al., Cell 57, 433, 1989).

Legends to the figures

Figure 1.

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Northern analysis of the novel estrogen receptor (ER β). Two different multiple tissue Northern blots (Clontech) were hybridised with a specific probe for ER β (see examples). Indicated are the human tissues the RNA originated from and the position of the size markers in kilobases (kb).

O Figure 2.

Histogram showing the 3- to 4-fold stimulatory effect of 17β-estradiol, estriol and estrone on the luciferase activity mediated by Enβ. An expression vector encoding ERβ was transiently transfected into CHO cells together with a reporter construct containing the rat oxytocin promoter in front of the firefly luciferase encoding sequence (see examples).

Figure 3.

Effect of 17β-estradiol (E2) alone or in combination with the anti-estrogen ICI-164384 (ICI) on ER α and ER β . Expression constructs for ER α (the classical ER) and ER β were transiently transfected into CHO cells together with the rat oxytocin promoter-luciferase reporter construct described in the examples. Luciferase activities were determined in triplicate and normalised for transfection efficiency by measuring β -galactosidase in the same lysate.

Figure 4.

Expression of ERα and ERβ in a number of cell lines determined by RT-PCR analysis (see examples). The cell lines used were derived from different tissues/cell types: endometrium (ECC1, Ishikawa, HEC-1A, RL95-2); osteosarcoma (SAOS-2, U2-OS, HOS, MG63); breast tumours (MCF-7, T47D), endothelium (HUV-EC-C, BAEC-1); smooth muscle (HISM, PAC-1, A7R5, A10, RASMC, CavaSMC); liver (HepG2); colon (CaCo2); and vagina (Hs-760T, SW-954).

All cell lines were human except for PAC-1, A7R5, A10 and RASMC which are of rat origin, BAEC-1 which is of bovine origin and CavaSMC which is of guinea pig origin

Figure 5.

and which is should be

Transactivation assay using stably transfected CHO cell lines expressing ER α or ER β together with the rat oxytocin-luciferase estrogen-responsive reporter (see examples for details). Hormone-dependent transactivation curves were determined for 17 β -estradiol and for Org4094. For the ER antagonist raloxifen, cells were treated with 2 x 10⁻¹⁰ mol/L 17 β -estradiol together with increasing concentrations of raloxifen. Maximal values of the responses were arbitrarily set at 100%.

Examples

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A. Molecular cloning of the novel estrogen receptor.

Two degenerate oligonucleotides containing inosines (I) were based on conserved regions of the DNA-binding domains and the ligand-binding domains of the human steroid hormone receptors.

Primer #1:

5'-GGIGA(C/T)GA(A/G)GC(A/T)TCIGGITG(C/T)CA(C/T)TA(C/T)GG-3'
(SEQ ID NO:7).

Primer #2:

5'-AAGCCTGG (C/G)A(C/T)IC(G/T)(C/T)TTIGCCCAI(C/T)TIAT-3' SEQ ID NO:8).

As template, cDNA from human EBV-stimulated PBLs (peripheral blood leukocytes) was used. One microgram of total RNA was reverse transcribed in a 20 µl reaction containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 4 mM MgCl2, 1 mM dNTPs (Pharmacia), 100 pmol random hexanucleotides (Pharmacia), 30 Units RNAse inhibitor (Pharmacia) and 200 Units M-MLV Reverse transcriptase (Gibco BRL). Reaction mixtures were incubated at 37°C for 30 minutes and heat-inactivated at 100°C for 5 minutes. The cDNA obtained was used in a 100 µl PCR reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin (w/v), 3% DMSO, 1 microgram of primer #1 and primer #2 and 2.5 Units of Amplitaq DNA polymerase (Perkin Elmer). PCR reactions were performed in the Perkin Elmer 9600 thermal cycler. The initial denaturation (4 minutes at 94°C) was followed by 35 cycles with the following conditions: 30 sec. 94°C, 30 sec. 45°C, 1 minute 72°C and after 7 minutes at 72°C the reactions were stored at 4°C. Aliquots of these reactions were analysed on a 1.5% agarose gel. Fragments of interest were cut out of the gel, reamplified using identical PCR-conditions and purified using Qiaex II (Qiagen). Fragments were cloned in the pCRII vector and transformed into bacteria using the TA-cloning kit (Invitrogen). Plasmid DNA was isolated for nucleotide sequence analysis using the Qiagen, plasmid midi protocol (Qiagen). Nucleotide sequence analysis was performed with the ALF automatic sequencer (Pharmacia) using a T7 DNA sequencing kit (Pharmacia) with vector-specific or fragment-specific primers.

One cloned fragment corresponded to a novel estrogen receptor (ER) which is closely related to the classical estrogen receptor. Part of the cloned novel estrogen receptor fragment (nucleotides 466 to 797 in SEQ ID 1) was amplified by PCR using oligonucleotide #3 TGTTACGAAGTGGGAATGGTGA (SEQ ID NO:9) and oligonucleotide #2 and used as a probe to screen a human testis cDNA library in \(\lambda\)gt11 (Clontech #HL1010b). Recombinant phages were plated (using Y1090 bacteria grown in LB medium supplemented with 0.2% maltose) at a density of 40.000 pfu (plaqueforming units) per 135 mm dish and replica filters (Hybond-N, Amersham) were made as described by the supplier. Filters were prehybridised in a solution containing 0.5 M phosphate buffer (pH 7.5) and 7% SDS at 65°C for at least 30 minutes. DNA probes were purified with Qiaex II (Qiagen), \(\frac{32}{2}\)P-labeled with a Decaprime kit (Ambion) and added to the prehybridisation solution. Filters were hybridised at 65°C overnight and then washed in 0.5 X SSC/0,1% SDS at 65°C. Two positive plaques were identified and could be shown to be identical. These clones were purified by rescreening one more time. A PCR reaction on the phage eluates with the \(\lambda\)gt11-specific primers #4: 5'-TTGACAC-CAGACCAACTGGTAATG-3' (SEQ ID NO:10) and #5: 5'-GGTGGCGACGACTCCTGGAGCCCG-3' (SEQ ID NO:11)

yielded a fragment of 1700 basepairs on both clones.

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Subsequent PCR reactions using combinations of a gene-specific primer #6: 5'-GTACACTGATTTGTAGCTGAC-3' (SEQ ID NO:12) with the \(\lambda\)glll primer #4 and gene-specific primer #7: 5'-CCATGATGATGTCCCTGACC-3' (SEQ ID NO:13) with \(\lambda\)gt11 primer primer #5 yielded fragments of approximately 450 bp and 1000 bp. respectively, which were cloned in the pCRII vector and used for nucleotide sequence analysis. The conditions for these PCR reactions were as described above except for the primer concentrations (200 ng of each primer) and the annealing temperature (60°C). Since in the cDNA clone the homology with the ER is lost abruptly at a site which corresponds to the exon 7/exon 8 boundary in the ER (between nucleotides 1247 and 1248 in SEQ ID NO:1), it was suggested that this sequence corresponds to intron 7 of the novel ER gene. For verification of the nucleotide sequences of this cDNA clone, a 1200 bp fragment was generated on the cDNA clone with \(\lambda\)gt11 primer #4 with a gene-specific primer #8 corresponding to the 3' end of exon 7: 5'-TCGCATGCCTGACGTGGGAC-3' (SEQ ID NO:14) using the proofreading *Pfu* polymerase (Stratagene). This fragment was also cloned in the pCRII vector and completely sequenced and was shown to be identical to the sequences obtained earlier.

To obtain nucleotide sequences of the novel ER downstream of exon 7, a degenerate oligonucleotide based on the AF-2 region of the classical ER (#9: 5'-GGC(C/G)TCCAGCATCTCCAG(C/G)A(A/G)GAG-3'; SEQ ID NO:15) was used together with the gene-specific oligonucleotide #10: 5'-GGAAGCTGGCTCACTTGCTG-3' (SEQ ID NO:16) using testis cDNA as template (Marathon ready testis cDNA, Clontech Cat #7414-1). A specific 220 bp fragment corresponding to nucleotides 1112 to 1332 in SEQ ID No. 1 was cloned and sequenced. Nucleotides 1112 to 1247 were identical to the corresponding sequence of the cDNA clone. The sequence downstream thereof is highly homologous with the corresponding region in the classical ER. In order to obtain sequences of the novel ER downstream of the AF-2 region, RACE (rapid amplification of cDNA ends) PCR reactions were performed using the Marathon-ready testis cDNA (Clontech) as template. The initial PCR was performed using oligonucleotide #11: 5'-TCTTGTTCTGGACAGGGATG-3' (SEQ ID NO:17) in combination with the AP1 primer provided in the kit. A nested PCR was performed on an aliquot of this reaction using oligonucleotide #10 (SEQ ID NO:16) in combination with the oligo dT primer provided in the kit. Subsequently, an aliquot of this reaction was used in a nested PCR using oligonucleotide #12: 5'-GCATGGAACATCTGCT-CAAC-3' (SEQ ID NO:18) in combination with the oligo dT primer. Nucleotide sequence analysis of a specific fragment that was obtained (corresponding to nucleotides 1256 to 1431 in SEQ ID NO 1) revealed a sequence encoding the carboxyterminus of the novel ER ligand-binding domain, including an F-domain and a translational stop codon and part of the 3' untranslated sequence which is not included in SEQ ID NO:1. The deduced amino acid sequence is shown in SEQ ID NO:5.

In order to investigate the possibility that the novel estrogen receptor had additional, upstream translation-initiation codons, RACE-PCR experiments were performed using Marathon-ready testis cDNA (Clontech Cat. # 7414-1). First a PCR was performed using oligonucleotide SEQ ID NO:12 (antisense corresponding to nucleotides 416-395 in SEQ ID NO:1) and AP-1 (provided in the kit). A nested PCR was then performed using oligonucleotide having SEQ ID NO:27 (antisense corresponding to nucleotides 254-231 in SEQ ID NO:1) with AP-2 (provided in the kit). From the smear that was obtained, the region corresponding to fragments larger than 300 basepairs was cut out, purified using the GenecleanII kit (Bio101) and cloned using the TA-cloning kit (Clontech). Colonies were screened by PCR using genespecific primers: SEQ ID NO:22 and SEQ ID NO:28. The clone containing the largest insert was sequenced. The nucleotide sequence corresponds to nucleotides 1 to 490 in SEQ ID NO:24. It is clear from this sequence that the first in-frame upstream translation initiation codon is present at position 77-79 in SEQ ID NO:24. Upstream of this translational startcodon an in-frame stop-codon is present (11-13 in SEQ ID NO:24). Consequently, the reading frame of the novel estrogen receptor is 530 amino acids (shown in SEQ ID NO:25) and has a calculated molecular mass of 59.234

To confirm the nucleotide sequences obtained by 5' RACE, human genomic clones were obtained and analysed. A human genomic library in λEMBL3 (Clontech HL1067J) was screened with a probe corresponding to nucleotides 1 to 416 in SEQ ID NO:1. A strongly hybridizing clone was plaque-purified and DNA was isolated using standard protocols (Sambrook et al. 1989). The DNA was digested with several restriction enzymes, electrophoresed on agarose gel and blotted onto Nylon filters. Hybridisation of the blot with a probe corresponding to the above-mentioned RACE fragment (nucleotides 1-490 in SEQ ID NO:24) revealed a hybridizing Sau3A fragment of approximately 800 basepairs. This fragment was cloned into the BamH1 site of pGEM3Z and sequenced. The nucleotide sequence contained one base difference which is probably a PCR-induced point mutation in the RACE fragment. Nucleotide 172 was a G residue in the 5'RACE fragment, but an A residue in several independent genomic subclones.

B. Identification of two splice variants of the novel estrogen receptor.

Rescreening of the testis cDNA library with a probe corresponding to nucleotides 918 to 1246 in SEQ ID No. 1 yielded two hybridizing clones, the 3' end of which were amplified by PCR (gene-specific primer #10: 5'-GGAAGCT-GGCTCACTTGCTG-3' (SEQ ID NO:16) together with primer #4, SEQ ID NO:10), cloned and sequenced. One clone

was shown to contain an alternative exon 8 (exon 8B) of the novel ER. In SEQ ID No. 2 the protein encoding part and the stopcodon of this splice variant are presented. As a consequence of the introduction of this exon through an alternative splicing reaction, the reading frame encoding the novel ER is immediately terminated, thereby creating a trun-

Screening of a human thymus cDNA library (Clontech HL1074a) with the probe corresponding to nucleotides 918 cation of the carboxyterminus of the novel ER (SEQ ID NO:6). to 1246 in SEQ ID No. 1, revealed another splice variant. The 3' end of one hybridizing clone was amplified using primer #10 (SEQ ID NO:16) with the λgt10-specific primer #13.5'-AGCAAGTTCAGCCTGTTAAGT-3' (SEQ ID NO:19). cloned and sequenced. The obtained nucleotide sequence upstream of the exon 7/exon 8 boundary was identical to the clones identified earlier. However, an alternative exon 8 (exon 8C) was present at the 3' end encoding two Cterminal amino acids followed by a stop-codon. The nucleotide sequence of the protein-encoding part of this splice variant is shown in SEQ ID NO:20, the corresponding protein sequence is SEQ ID NO:21.

These two variants of the novel estrogen receptor do not contain the AF-2 region and therefore probably lack the ability to modulate transcription of target genes in a ligand-dependent fashion. However, the variants potentially could interfere with the functioning of the wild-type classical ER and/or the wild-type novel ER, either by heterodimerization or by occupying estrogen response elements or by interactions with other transcription factors. A mutant of the classical ER (ER1-530) has been described which closely resembles the two variants of the novel estrogen receptor described above. ER1-530 has been shown to behave as a dominant-negative receptor i.e. it can modulate the intracellular activity of the wild type ER (Ince et al, J. Biol. Chem. 268, 14026-14032, 1993).

Human multiple tissue Northern blots (MTN-blots) were purchased from Clontech and prehybridized for at least 1 C. Northern blot analysis. hour at 65°C in 0.5 M phosphate buffer pH 7.5 with 7% SDS. The DNA fragment that was used as a probe (corresponding to nucleotides 466 to 797 in SEQ ID No. 1) was ³²P-labeled using a labelling kit (Ambion), denatured by boiling and added to the prehybridisation solution. Washing conditions were: 3X SSC at room temperature, followed by 3 X SSC at 65°C, and finally 1 X SSC at 65°C. The filters were than exposed to X-ray films for one week. Two transcripts of approximately 8 kb and 10 kb were detected in thymus, spleen, ovary and testis. In addition, a 1.3 kb transcript was detected in testis.

D. RT-PCR analysis of expression of ERa and ER β in cell lines.

RNA was isolated from a number of human and animal cell lines using RNAzol B (Cinna/Biotecx). cDNA was made using 2.5 microgram of total RNA using the Superscript II kit (BRL) following the manufacturers instructions. A portion of the cDNA was used for specific PCR amplifications of fragments corresponding either to mRNA encoding the ER or to the novel estrogen receptor. (It should be emphasized that the primers used are based on human and rat sequences, whereas some of the cell lines were not rat or human, see legend of Figure 4). Primers used were for ERa: sense 5'-GATGGGCTTACTGACCAACC-3' and antisense 5'-AGATGCTCCATGCCTTTG-3' generating a 548 base pair fragment corresponding to part of the LBD. For ERβ: sense 5'- TTCACCGAGGCCTCCATGATG-3' and antisense 5'-CAGATGTTCCATGCCCTTGTT-3' generating a 565 base pair fragment corresponding to part of the LBD. The PCR samples were analysed on agarose which were blotted onto Nylon membranes. These blots were hybridised with ³²Plabeled PCR fragments generated with the above-mentioned primers on ER α and ER β plasmid DNA using standard experimental procedures (Sambrook et al, 1989).

E. Ligand-dependent transcription activation by the novel estrogen receptor protein.

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Chinese Hamster Ovary (CHO K1) cells were obtained from ATCC (CCL61) and maintained at 37°C in a humidified Cell culture atmosphere (5% CO₂) as a monolayer culture in fenolred-free M505 medium. The latter medium consists of a mixture (1:1) of Dulbecco's Modified Eagle's Medium (DMEM, Gibco 074-200) and Nutrient Medium F12 (Ham's F12, Gibco 074-1700) supplemented with 2.5 mg/ml sodium carbonate (Baker), 55 μ g/ml sodium pyruvate (Fluka), 2.3 μ g/ml β mercaptoethanol (Baker), 1.2 μg/ml ethanolamine (Baker), 360 μg/ml L-glutamine (Merck), 0.45 μg/ml sodium selenite (Fluka), 62.5 μg/ml penicillin (Mycopharm), 62.5 μg/ml streptomycin (Serva), and 5% charcoal-treated bovine calf serum (Hyclone).

Recombinant vectors

The ERß-encoding sequence as presented in SEO ID No. 1 was amplified by PCR using oligonucleotides 5'-

CTTGGATCCATAGCCCTGCTGTGATGAATTACAG-3' (SEQ ID NO:22 underlined is the translation initiation codon) in combination with 5'-GATGGATCCTCACCTCAGGGCCAGGCGTCACTG-3' (SEQ ID NO:23) (underlined is the translation stopcodon, antisense). The resulting BamH1 fragment (approximately 1450 base pairs) were then cloned in the mammalian cell expression vector pNGV1 (Genbank accession No. X99274).

An expression construct encoding the ERβ reading frame as presented in SEQ ID NO:24 was made by replacing a BamH1-Msc1 fragment (nucleotides 1-81 in SEQ ID No. 1) by a BamH1-Msc1 fragment corresponding to nucleotides 77-316 in SEQ ID No. 24. The latter fragment was made by PCR with SEQ ID NO:26 in combination with SEQ ID NO: 28 using the above mentioned 5' RACE fragment.

The reporter vector was based on the rat oxytocin gene regulatory region (position -363/+16 as a HindIII/ Mbol fragment; R.Ivell, and D.Richter. Proc.Natl. Acad.Sci.USA 81, 2006-2010, 1984) linked to the firefly luciferase encoding sequence: the regulatory region of the oxytocin gene was shown to possess functional estrogen hormone response elements *in vitro* for both the rat (R.Adan *et al.*, Biochem.Biophys.Res.Comm. 175, 117-122, 1991) and the human (S. Richard and H.Zingg, J.Biol.Chem. 265, 6098-6103, 1990).

Transient transfection

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 1×10^5 CHO cells were seeded in 6-wells Nunclon tissue culture plates and DNA was introduced by use of lipofectin (Gibco BRL). Hereto, the DNA (1 μg of both receptor and reporter vector in 250 μL Optimem, Gibco BRL) was mixed with an equal volume of lipofectin reagent (7 μL in 250 μL Optimem, Gibco) and allowed to stand at room temperature for 15 min. After washing the cells twice with serum-free medium (M505) new medium (500 μL Optimem, Gibco) was added to the cells followed by the dropwise addition of the DNA-tipofectin mixture. After incubation for a 5 hour period at 37°C cells were washed twice with fenoIred-free M505 + 5% charcoal-treated bovine calf serum and incubated overnight at 37°C. After 24 hours hormones were added to the medium (10^{-7} mol/L). Cell extracts were made 48 hours posttransfection by the addition of 200 μL lysisbuffer (0.1 M phosphate buffer pH7.8, 0.2% Triton X-100). After incubation for 5 min at 37°C the cell suspension was centrifuged (Eppendorf centrifuge, 5 min) and 20 μL sample was added to 50 μL luciferase assay reagent (Promega). Light emission was measured in a luminometer (Berthold Biolumat) for 10 sec at 562 nm.

Stable transfection of the novel estrogen receptor.

The expression plasmid encoding full-length ER β 1-530 (see above) was stably transfected in CHO K1 cells as previously described (Theunissen *et al.*, J. Biol. Chem. 268, 9035-9040, 1993). Single cell clones that were obtained this way were screened by transient transfection of the reporter plasmid (rat oxytocin-luciferase) as described above. Selected clones were used for a second stable transfection of the rat oxytocin-luciferase reporter plasmid together with the plasmid pDR2A which contains a hygromycine resitance gene for selection. Single cell clones obtained were tested for a response to 17 β -estradiol. Subsequently, a selected single cell clone was used for transactivation studies. Briefly, cells were seeded in 96-wells at (1.6x10⁴ cells per well). After 24 hours different concentrations of hormone were diluted in medium and added to the wells. For antagonistic experiments, 2x10⁻¹⁰ M. 17 β -estradiol was added to each well and different concentrations of antagonists were added. Cells were washed once with PBS after a 24 hour incubation and then lysed by the addition of 40 microliter lysis buffer (see above). Luciferase reagent was added (50 microliter) to each well and light emission was measured using the Topcount (Packard).

Results.

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A comparison of the two expression constructs (SEQ ID NO:1 and SEQ ID NO:24) in transient transfections in CHO cells showed identical transactivation in response to a number of agonists and antagonists. CHO cells transiently transfected with ERβ expression vector and a reporter plasmid showed a 3 to 4 fold increase in luciferase activity in response to 17β-estradiol as compared to untreated cells (see Figure 2). A similar transactivation was obtained upon treatment with estriol and estrone. The results indicate not only that the novel ER (ERβ) can bind estrogen hormones but also that the ligand-activated receptor can bind to the estrogen-response elements (EREs) within the rat oxytocin promoter and activate transcription of the luciferase reporter gene. Figure 3 shows that in an independent similar experiment 10⁻⁹ mol/L 17β-estradiol gave an 18-fold stimulation with ERα and a 7-fold stimulation with ERβ. In addition, the antiestrogen ICI-164384 was shown to be an antagonist for both ERα and ERβ when activated with 17β-estradiol, whereas the antagonist alone had no effect. In this experiment 0.25 μg β-galactosidase vector was co-transfected in order to normalize for differences in transfection efficiency.

Transactivation studies performed on stably transfected ER α and ER β cell lines gave similar absolute luciferase values. The curves for 17 β -estradiol are very similar and show that half-maximal transactivation is reached with lower concentrations of hormone on ER α as compared to ER β (Figure 5). For Org4094 this is also the case however, the

effect observed is much more pronounced. The curves for raloxifen show that the potency of this antagonist to block transactivation on $\mathsf{ER}\alpha$ is greater compared to its potency to block $\mathsf{ER}\beta$ transactivation

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SEQUENCE LISTING

English Startes and Startes

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| | (1) GENERAL INFORMATION: |
|----|--|
| | (i) APPLICANT: |
| 10 | (A) NAME: Akzo nobel n.v. |
| | (B) STREET: Velperweg 76 |
| | (C) CITY: Arnhem |
| 15 | (E) COUNTRY: The Netherlands |
| | (F) POSTAL CODE (ZIP): 6824 BM |
| | (G) TELEPHONE: 0412-666379 |
| | (H) TELEFAX: 0412-650592 |
| 20 | (I) TELEX: 37503 akpha nl |
| | (ii) TITLE OF INVENTION: Novel estrogen receptor |
| 25 | (iii) NUMBER OF SEQUENCES: 28 |
| | (iv) COMPUTER READABLE FORM: |
| 30 | (A) MEDIUM TYPE: Floppy disk |
| | (B) COMPUTER: IBM PC compatible |
| | (C) OPERATING SYSTEM: PC-DOS/MS-DOS |
| | (D) SOFTMARE: PatentIn Release #1.0, Version #1.30 (EPO) |
| 35 | |
| | (2) INFORMATION FOR SEQ ID NO: 1: |
| 40 | (i) SEQUENCE CHARACTERISTICS: |
| | (A) LENGTH: 1434 base pairs |
| • | (B) TYPE: nucleic acid |
| 45 | (C) STRANDEDNESS: double |
| | (D) TOPOLOGY: linear |
| | (ii) MOLECULE TYPE: cDNA |
| 50 | |
| | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1: |

| | ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGGAAG GTGGGCCTGG TCGGCAGACC | 60 |
|----|---|-----------|
| i | ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC | 120 |
| | CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA | 180 |
| 0 | GAACACACCT TACCTGTAAA CAGAGAGACA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC | 240 |
| 5 | GCCAGCCCTG TTACTGGTCC AGGTTCAAAG AGGGATGCTC ACTTCTGCGC TGTCTGCAGC | 300 |
| J | GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT | 360 |
| 20 | AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC | 420 |
| | GATAAAAACC GGCGCAAGAG CTGCCAGGCC TGCCGACTTC GGAAGTGTTA CGAAGTGGGA | 480 |
| 25 | ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA | 540 |
| | AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC | 600 |
| 30 | CGAGTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG | 660 |
| | GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC | 720 |
| 35 | ATGATGATGT CCCTGACCAA GTTGGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC | 780 |
| 40 | AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTCGACC AAGTGCGGCT CTTGGAGAGC | 840 |
| | TGTTGGATGG AGGTGTTAAT GATGGGGGTG ATGTGGCGCT CAATTGACCA CCCCGGCAAG | 900 |
| 45 | CTCATCTTTG CTCCAGATCT TGTTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT | 960 |
| | CTGGAAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA | 1020 • |
| 50 | CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCTG | 1080 |
| | GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGTG | 1140 |

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| | ACCGATGCTT TGGTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCCAGCA GCAATCCATG | 1200 |
|----|--|-------------|
| 5 | CGCCTGGCTA ACCTCCTGAT GCTCCTGTCC CACGTCAGGC ATGCGAGTAA CAAGGGCATG | 1260 |
| | GAACATCTGC TCAACATGAA GTGCAAAAAT GTGGTCCCAG TGTATGACCT GCTGCTGGAG | 1320 |
| 10 | ATGCTGAATG CCCACGTGCT TCGCGGGTGC AAGTCCTCCA TCACGGGGTC CGAGTGCAGC | 1380 |
| | CCGGCAGAGG ACAGTAAAAG CAAAGAGGGC TCCCAGAACC CACAGTCTCA GTGA | 1434 |
| 15 | (2) INFORMATION FOR SEQ ID NO: 2: | |
| 20 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1251 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear | |
| 30 | (ii) MOLECULE TYPE: cDNA | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2: | |
| 35 | ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGGAAG GTGGGCCTGG TCGGCAGACC | 60 |
| | ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC | 120 |
| 40 | CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA | 180 |
| | CAACACACCT TACCTGTAAA CAGAGAGACA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC | 240 |
| 45 | GCCAGCCCTG TTACTGGTCC AGGTTCAAAG AGGGATGCTC ACTTCTGCGC TGTCTGCAGC | 300 |
| 50 | GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT | *360 |
| 20 | AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC | 420 |
| | GATAAAAACC GGCGCAAGAG CTGCCAGGCC TGCCGACTTC GGAAGTGTTA CGAAGTGGGA | 480 |

| | ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA | 540 |
|----|---|------|
| 5 | ACTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC | 600 |
| | CCACTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG | 660 |
| 10 | CACCCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC | 720 |
| | ATCATGATGT CCCTGACCAA GTTGGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC | 780 |
| 15 | AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTCGACC AAGTGCGGCT CTTGGAGAGC | 840 |
| 20 | TGTTGGATGG AGGTGTTAAT GATGGGGCTG ATGTGGCGCT CAATTGACCA CCCCGGCAAG | 900 |
| | CTCATCTTTG CTCCAGATCT TGTTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT | 960 |
| 25 | CTGGAAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA | 1020 |
| | CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCTG | 1080 |
| 30 | GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGTG | 1140 |
| | ACCGATGCTT TGGTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCCAGCA GCAATCCATG | 1200 |
| 35 | CGCCTGGCTA ACCTCCTGAT GCTCCTGTCC CACGTCAGGC ATGCGAGGTG A | 1251 |
| 40 | (2) INFORMATION FOR SEQ ID NO: 3: | |
| | (1) SEQUENCE CHARACTERISTICS: | |
| | (A) LENGTH: 66 amino acids | |
| 4 | (B) TYPE: amino acid | |
| | (C) STRANDEDNESS: single | • |
| | (D) TOPOLOGY: linear | • |
| | (ii) MOLECULE TYPE: peptide | |

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| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3: |
|----|--|
| 5 | Cys Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp 1 5 10 15 |
| 10 | Ser Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His 20 25 30 |
| 15 | Asn Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn 35 40 45 |
| | Arg Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val 50 55 60 |
| 20 | Gly Met 65 |
| 25 | (2) INFORMATION FOR SEQ ID NO: 4: |
| 30 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 233 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single |
| 35 | (ii) MOLECULE TYPE: peptide |
| 40 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4: |
| 45 | Leu Val Leu Thr Leu Leu Glu Ala Glu Pro Pro His Val Leu Ile Ser 1 5 10 15 |
| 50 | Arg Pro Ser Ala Pro Phe Thr Glu Ala Ser Met Met Net Ser Leu Thr 20 25 30 |
| | Lys Leu Ala Asp Lys Glu Leu Val His Met Ile Ser Trp Ala Lys Lys 35 40 45 |

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| | Ile | Pro 50 | Gly | Phe | Val | Glu | Leu 55 | Ser | Leu | Phe | Asp | Gln 60 | Val | Arg | Leu | Leu |
|----|------------|------------|------------|------------|-----------|------------|-------------|---------------------|------------|-----------|------------|------------|-------------|------------------|-----------|-----------|
| 5 | Glu 65 | Ser | Суз | Trp | Met | Glu 70 | Val | Leu | Met | Met | Gly 75 | Leu | Met | Trp | Arg | Ser 80 |
| 10 | Ile | Asp | His | Pro | Gly 85 | Lys | Leu | Ile | Phe | Ala 90 | Pro | Азр | Leu | Val | Leu 95 | Asp |
| 15 | Arg | Asp | Glu | Gly 100 | Lys | Cys | Val | Glu | Gly 105 | Ile | Leu | Glu | Ile | Phe 110 | Asp | Met |
| 20 | Leu | Leu | Ala 115 | | Thr | Ser | Arg | Phe 120 | | Glu | Leu | Lys | Leu 125 | Gln | His | Lys |
| 25 | Glu | Tyr 130 | | Сув | Val | Lys | Ala 135 | | Ile | Leu | Leu | Asn 140 | Ser | Ser | Met | Tyr |
| 30 | Pro 145 | | Val | Thr | : Ala | Thr 150 | | Asp | Ala | Asp | Ser 155 | Ser | Arg | Lys | Leu | 160 |
| 30 | His | Let | ı Lev | ı Ası | 165 | | LThi | c Asp | Ala | 170 | | Tr | Val | . Ile | 175 | Lys |
| 35 | Sei | c Gl | y Il | e Se 18 | | r Gl | n Gl | n Gl | n Se: | | t Arg | j Le | u Ala | 19 ⁰ | n Lei | ı Leu |
| 40 | Ме | t Le | u Le 19 | | r Hi | s Va | l Ar | g Hi 20 | | a Se | r As: | n Ly | s Gl 20 | у Ме 5 | t Gl | u His |
| 45 | Le | u Le 21 | | in Me | et Ly | s Cy | | / s As 15 | sn Va | al Va | ıl Pr | o Va 22 | 11 Ty 20 | r As | p Le | u Leu |
| 50 | Le 22 | | Lu Me | et L | eu As | | la H: 30 | is V | al L | eu | | | | | | |
| (3 | 2) IN | FORM | ATIO | n Fo | R SE | Q ID | NO: | 5: | | | | | | | | |

(i) SEQUENCE CHARACTERISTICS:

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| | (A) LENGTH: 477 amino acids |
|----|--|
| 5 | (B) TYPE: amino acid |
| | (C) STRANDEDNESS: single |
| | (D) TOPOLOGY: unknown |
| 10 | (ii) MOLECULE TYPE: protein |
| 15 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5: |
| 20 | Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly Gly Pro 1 10 15 |
| 25 | Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro Gly His 20 25 30 |
| 30 | Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Tyr Ala Glu 35 40 45 |
| 30 | Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu His Thr Leu 50 55 60 |
| 35 | Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly Asn Arg Cys 65 70 75 80 |
| 40 | Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala His Phe Cys 85 90 95 |
| 45 | Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly Val Trp Ser 100 105 110 |
| 50 | Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln Gly His Asn 115 120 125 |
| 55 | Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp Lys Asn Arg 130 135 140 |

 $\mathcal{L}_{i}^{(n)}(t,\mathbf{z}) = \operatorname{det}_{i}^{(n)}(t,\mathbf{z})^{-\frac{1}{2}}$

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| | Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Tyr Glu Val Gly 145 150 155 160 |
|----|---|
| 5 | Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Tyr Arg Leu Val 165 170 175 |
| 10 | Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys Ala Gly Lys Ala 180 185 190 |
| 15 | Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu Leu Leu Leu Asp 205 |
| 20 | Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu Glu Ala Glu Pro 210 215 220 |
| | Pro His Val Leu Ile Ser Arg Pro Ser Ala Pro Phe Thr Glu Ala Ser 225 230 235 240 |
| 25 | Met Met Met Ser Leu Thr Lys Leu Ala Asp Lys Glu Leu Val His Met 255 245 |
| 30 | Ile Ser Trp Ala Lys Lys Ile Pro Gly Phe Val Glu Leu Ser Leu Phe 260 265 270 |
| 35 | Asp Gln Val Arg Leu Leu Glu Ser Cys Trp Met Glu Val Leu Met Met 285 |
| 40 | Gly Leu Met Trp Arg Ser Ile Asp His Pro Gly Lys Leu Ile Phe Ala 290 295 300 |
| 45 | Pro Asp Leu Val Leu Asp Arg Asp Glu Gly Lys Cys Val Glu Gly Ile 305 310 315 |
| | Leu Glu Ile Phe Asp Met Leu Leu Ala Thr Thr Ser Arg Phe Arg Glu 325 330 335 |
| | Leu Lys Leu Gln His Lys Glu Tyr Leu Cys Val Lys Ala Met Ile Leu 340 345 350 |

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| | Leu Asn Ser Ser Met Tyr Pro Leu Val Thr Ala Thr Gln Asp Ala Asp 355 360 365 |
|----|--|
| 5 | Ser Ser Arg Lys Leu Ala His Leu Leu Asn Ala Val Thr Asp Ala Leu 370 375 380 |
| 10 | Val Trp Val Ile Ala Lys Ser Gly Ile Ser Ser Gln Gln Gln Ser Het 385 390 395 400 |
| 15 | Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His Ala Ser 405 410 415 |
| 20 | Asn Lys Gly Met Glu His Leu Leu Asn Met Lys Cys Lys Asn Val Val 420 425 430 |
| | Pro Val Tyr Asp Leu Leu Leu Glu Met Leu Asn Ala His Val Leu Arg 435 440 445 |
| 25 | Gly Cys Lys Ser Ser Ile Thr Gly Ser Glu Cys Ser Pro Ala Glu Asp 450 455 460 |
| 30 | Ser Lys Ser Lys Glu Gly Ser Gln Asn Pro Gln Ser Gln 475 |
| 35 | (2) INFORMATION FOR SEQ ID NO: 6: |
| | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 416 amino acids |
| 40 | (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: unknown |
| 45 | (ii) MOLECULE TYPE: protein |
| 50 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: |
| 55 | Met Asn Tyr Ser Ile Pro Ser Asn Val Thr Asn Leu Glu Gly Gly Pro |
| | |

| | 10 | 15 |
|------------|--|-----------------------|
| | 1 5 | |
| | Gly Arg Gln Thr Thr Ser Pro Asn Val Leu Trp Pro Thr Pro | Gly His |
| 5 | 20 | |
| | Leu Ser Pro Leu Val Val His Arg Gln Leu Ser His Leu Ty | r Ala Glu |
| 10 | 35 | |
| | Glu Ala Arg Ser Leu Glu Hi | is Thr Leu |
| | Pro Gln Lys Ser Pro Trp Cys Glu Ala Arg Ser Leu Glu Hi | |
| 15 | Arg Lys Val Ser Gly A | _{an} Arg Cys |
| | Pro Val Asn Arg Glu Thr Leu Lys Arg Lys Val Ser Gly A 75 | 80 |
| | a Avg Asp Ala E | lis Phe Cys |
| 20 | Ala Ser Pro Val Thr Gly Pro Gly Ser Lys Arg Asp Ala E | 95 |
| | 85 | |
| | are for Gly | Val Trp Ser |
| 25 | Ala Val Cys Ser Asp Tyr Ala Ser Gly Tyr His Tyr Gly | 110 |
| 25 | 100 105 | |
| | an Tle Gln | Gly His Asn |
| | Cys Glu Gly Cys Lys Ala Phe Phe Lys Arg Ser Ile Gln | _ |
| 30 | 115 120 | |
| | | Lvs Asn Arg |
| | Asp Tyr Ile Cys Pro Ala Thr Asn Gln Cys Thr Ile Asp | |
| | 135 | |
| 35 | 130 | - Glu Val Gly |
| | Arg Lys Ser Cys Gln Ala Cys Arg Leu Arg Lys Cys Ty | 160 |
| | 130 | |
| | 145 | ava Teu Val |
| 40 | Met Val Lys Cys Gly Ser Arg Arg Glu Arg Cys Gly Ty | 175 |
| | 165 | |
| | Arg Arg Gln Arg Ser Ala Asp Glu Gln Leu His Cys A | la Gly Lys Ala |
| 45 | Arg Arg Gln Arg Ser Ala Asp Glu | 190 |
| | 180 | |
| | Lys Arg Ser Gly Gly His Ala Pro Arg Val Arg Glu | Leu Leu Leu Asp |
| 5 0 | Lys Arg Ser Gly Gly His Ala Pro Alg | 205 |
| | 195 | |
| | - val Leu Thr Leu Leu | Glu Ala Glu Pro |
| | Ala Leu Ser Pro Glu Gln Leu Val Leu Thr Leu Leu | |
| 55 | | |

| | | 220 | |
|-----|-------------------------------|---|-------------|
| | 215 | | |
| | 210 | | Ser |
| | Pro His Val Leu Ile Ser Arg P | ro Ser Ala Pro Phe Thr Giu Ala | 240 |
| | Pro His Val Leu Ile Ser Arg : | 235 | 240 |
| 5 | 230 | | |
| J | 225 | eu Ala Asp Lys Glu Leu Val Hi 250 250 | s Met |
| | mu Tus I | eu Ala Asp Lys Glu Leu Val | .5 |
| | Met Met Met Ser Leu Thi Bys | 250 | • |
| | | | |
| 10 | | Glu Leu Ser Le | au Phe |
| | al Tue Lys Ile | Pro Gly Phe Val Glu Leu Ser Le 265 270 | |
| | Ile Ser Trp Ala by | 265 | |
| | | | |
| ar. | | Ser Cys Trp Met Glu Val Leu M 280 285 | et Met |
| 15 | and Ard Leu Leu Glu | Ser Cys Trp Acc 285 | |
| | Asp Gln var Aly | 280 | |
| | 275 | , | nha Ala |
| | | his Pro Gly Lys Leu Ile | rne Al- |
| 20 | Glas Leu Met Trp Arg Ser Ile | e Asp His Pro Gly Lys Leu Ile 1 5 300 | |
| | | | |
| | 290 | | Gly Ile |
| | X | a Asp Glu Gly Lys Cys Val Glu | 320 |
| _ | Pro Asp Leu Val Leu Asp A | g Asp Glu Gly Lys Cys Val Glu 315 | 324 |
| 25 | 9. * | | |
| | 305 | eu Leu Ala Thr Thr Ser Arg Phe 330 | Arg Glu |
| | nhe len Met L | eu Leu Ala Thr Thr Ser | 335 |
| | Leu Glu Ile Pile PDF | 330 | |
| 30 | 347 | | |
| | | Glu Tyr Leu Cys Val Lys Ala Met 345 | f IIe ben |
| | Leu Lys Leu Gln His Lys | 31u Tyr 202 -3 | 0 |
| | 340 | | |
| 35 | ••• | Pro Leu Val Thr Ala Thr Gln As | n Ala Asp |
| 33 | n b Mur | Pro Leu Val Thr Ala Thr Gin Al | , P |
| | Leu Asn Ser Ser Met 191 | 365 | |
| | 255 | | |
| | | His Leu Leu Asn Ala Val Thr A | sp Ala Leu |
| 40 | and Ive Leu Ale | His Leu Leu Ash Ala | |
| | Ser Ser Arg 115 | 375 | |
| | | | |
| | | s Ser Gly Ile Ser Ser Gln Gln (| Gin Ser Hee |
| 45 | Tro Val Ile Ala Ly | s Ser Gry 112 0- | 400 |
| • | | | |
| | 385 | eu Met Leu Leu Ser His Val Arg 410 | His Ala Arg |
| | * | an Met Leu Leu Ser His Val Arg | 415 |
| | Arg Leu Ala Asn Leu L | 410 | #73 |
| 50 | 405 | | |
| | | | |
| | | | |

| | | EP 0 798 378 A2 | |
|--|--------|--|----|
| A STATE OF THE STA | | | |
| 1947 1944 | | | |
| | | mp seo ID NO: 7: | |
| | (2) IN | FORMATION FOR SEQ ID NO: 7: | |
| And the second of the second o | · | CUARACTERISTICS: | |
| A to the first of | 5 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs | |
| | | (A) LENGTH: 25 DEC. (B) TYPE: nucleic acid | |
| • • | | (B) TYPE: nucrosis; both | |
| • • | | (C) STRANDEDNESS: both | |
| | 10 | (D) TOPOLOGY: unknown | |
| | | on. cona | |
| | | (ii) MOLECULE TYPE: CDNA | |
| | | | |
| | 15 | | |
| | | TERRIPTION: SEQ ID NO: 7: | |
| | | (x1) SEQUENCE DESCRIPTION: SEQ ID NO: 7: | 29 |
| | 20 | VCAYTAYGG | |
| | ec. | igaygarg cwtciggitg ycaytaygg | |
| | 60 | - TD NO: 8: | |
| | t s | 2) INFORMATION FOR SEQ ID NO: 8: | |
| | 25 | THE PROPERTY OF THE PROPERTY O | |
| | | (i) SEQUENCE CHARACTERISTICS: | |
| | | | |
| | | (B) TYPE: nucleic acid | |
| • | 30 | (B) TYPE: ndoses (C) STRANDEDNESS: single | |
| | | (D) TOPOLOGY: linear | |
| | | | |
| | | (ii) MOLECULE TYPE: CINA | |
| | 35 | • | |
| | | | |
| | | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: | |
| | . 40 | (xi) SEQUENCE DESCRIPTION. | 29 |
| | . 40 | TATTAT | |
| | • | AAGCCTGGSA YICKYTTIGC CCAIYTIAT | |
| | · | Armoss. 9: | • |
| ** | 45 | (2) INFORMATION FOR SEQ ID NO: 9: | • |
| | | (4) | |
| | | (i) SEQUENCE CHARACTERISTICS: | |
| | | | |
| | 50 | miclel about | |
| | | CMPANDEDNESS: SILVE | |
| | | (D) TOPOLOGY: linear | |
| | | | |
| | cc | | |

| The state of the s | | o78 A2 | |
|--|-----------|---|----|
| | | EP 0 798 378 A2 | |
| A CONTRACTOR OF THE SECOND SEC | | | |
| and the second second second | | | |
| | 140 | LECULE TYPE: CDNA | |
| | (ii) AO | | |
| A State Charles of Recorded 197 | 5 | | |
| | | SPO ID NO: 9: | |
| | (xi) | SEQUENCE DESCRIPTION: SEQ ID NO: 9: | 22 |
| | | | |
| | TGTTACGAA | G TGGGAATGGT GA | |
| | ,- | SEO ID NO: 10: | |
| | (2) INFOR | RMATION FOR SEQ ID NO: 10: | |
| | 15 | CUARACTERISTICS: | |
| | (i) | | |
| | | nucleic wo | |
| | 20 | SEPANDEDNESS: | |
| | | (D) TOPOLOGY: linear | |
| | | | |
| | | 1) MOLECULE TYPE: cDNA | |
| | 25 (1) | 11 | |
| | | | |
| | | TOTTON: SEQ ID NO: 10: | |
| | 30 (: | xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: | 24 |
| | | | |
| | TTGAC | CACCAG ACCAACTGGT AATG | |
| | 35 | INFORMATION FOR SEQ ID NO: 11: | |
| | (2) | INFORMATION FOR - | |
| | | CUADACTERISTICS: | |
| | | | |
| | 40 | | |
| | | CEPANDEDNESS: | |
| | | (D) TOPOLOGY: linear | |
| | 45 | | 1 |
| | | (11) MOLECULE TYPE: cDNA | |
| | | (4-) | |
| | 50 | | |
| | 50 | ORSCRIPTION: SEQ ID NO: 11: | |
| | | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: | |
| | • | | |
| | 55 | | |

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| GGT | GGCGACG ACTCCTGGAG CCCG | |
|------------|--|----|
| 5 (2) | INFORMATION FOR SEQ ID NO: 12: | |
| 10 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single | |
| | (C) STRANDERING (D) TOPOLOGY: linear | |
| 15 | (ii) MOLECULE TYPE: CDNA | |
| 20 | (x1) SEQUENCE DESCRIPTION: SEQ ID NO: 12: | 22 |
| 0.5 | GTACACTGAT TTGTAGCTGG AC | |
| 25 | (2) INFORMATION FOR SEQ ID NO: 13: | |
| 30 35 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
| | (ii) MOLECULE TYPE: CDNA | |
| 40 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13: | 20 |
| 45 | (xi) SEQUENCE DEC | • |
| 5 <i>C</i> | (2) INFORMATION FOR SEQ ID NO: 14: | |
| | | |

And the second second second second

| | (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
|----|--|----|
| 5 | (ii) MOLECULE TYPE: cDNA | |
| 10 | SPO ID NO: 14: | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14: | 20 |
| 15 | TCGCATGCCT GACGTGGGAC | |
| | (2) INFORMATION FOR SEQ ID NO: 15: | |
| 20 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs | |
| 25 | (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
| 30 | (ii) MOLECULE TYPE: cDNA | |
| 35 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15: | 24 |
| | GGCSTCCAGC ATCTCCAGSA RCAG | |
| 40 | (2) INFORMATION FOR SEQ ID NO: 16: | |
| 45 | (B) TYPE: nucleic acid | |
| £ | (C) STRANDEDNESS: single (D) TOPOLOGY: linear | : |
| | (ii) MOLECULE TYPE: cDNA | |

 $\sup_{n \in \mathbb{N}} \frac{p(n)}{2} \leq \frac{p(n)}{2} \leq \frac{p(n)}{2}$

The last of the property of And the second s

| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16: | |
|----|---|-------------|
| | (xi) SEQUENCE DESCRIPTION | 20 |
| 5 | GGAAGCTGGC TCACTTGCTG | |
| | (2) INFORMATION FOR SEQ ID NO: 17: | |
| 10 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid | |
| 15 | (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
| | (11) MOLECULE TYPE: CDNA | |
| 20 | · | |
| 25 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO:-17: | 20 |
| | TCTTGTTCTG GACAGGGATG | |
| 30 | (2) INFORMATION FOR SEQ ID NO: 18: | |
| | (i) SEQUENCE CHARACTERISTICS: | |
| 35 | (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
| 40 | (ii) MOLECULE TYPE: cDNA | |
| | | |
| 45 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18: | * 20 |
| | GCATGGAACA TCTGCTCAAC | |
| 5 | (2) INFORMATION FOR SEQ ID NO: 19: | |
| · | (i) SEQUENCE CHARACTERISTICS: | |

Section 1997 - The Control of the Co

| (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
|--|-----|
| (ii) MOLECULE TYPE: cDNA | |
| 0 | |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19: | |
| AGCAAGTTCA GCCTGTTAAG T | |
| 20 (2) INFORMATION FOR SEQ ID NO: 20: | |
| (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1257 base pairs | |
| 25 (B) TYPE: nucleic double | |
| (D) TOPOLOGY: linear | |
| (ii) MOLECULE TYPE: cDMA | |
| (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: | 60 |
| (xi) SEQUENCE DESCRIPTION: SEQ TO THE ACCTTGGAAG GTGGGCCTGG TCGGCAGACC ATGAATTACA GCATTCCCAG CAATGTCACT AACTTGGAAG GTGGGCCTGG TCGGCAGACC ATGAATTACA GCATTCCCAG CAATGTCACT CCGCACCTTT CTCCTTTAGT GGTCCATCGC | 120 |
| ATGAATTACA GCATTCCCAG CAATGTCACT ARCTION ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT GGTCCATCGC ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGCACCTTT CTCCTTTAGT AGATCGCTA | 180 |
| ACAAGCCCAA ATGTGTTGTG GCCAACACCT GGGGTCAAGC AAGATCGCTA 45 CAGTTATCAC ATCTGTATGC GGAACCTCAA AAGAGTCCCT GGTGTGAAGC AAGATCGCTA CTGAAAAGGA AGGTTAGTGG GAACCGTTGC | 240 |
| GAACACACCT TACCTGTAAA CAGAGAGACA CIGGGTGCTC ACTTCTGCGC TGTCTGCAGC | 300 |
| GCCAGCCCTG TTACTGGTCC AGGTTCAAAG AGGGTGTAA GGCCTTTTTT GATTACGCAT CGGGATATCA CTATGGAGTC TGGTCGTGTG AAGGATGTAA GGCCTTTTTT | 360 |
| GATTACGCAT CGGGATATCA CTATGGAGTC | |
| | |

| CHCTACAATC | 120 |
|--|------------|
| AAAAGAAGCA TTCAAGGACA TAATGATTAT ATTTGTCCAG CTACAAATCA GTGTACAATC | |
| TTCAAGGACA TAATGATTAT ATTI | 480 |
| AAAAGAAGCA TTCAAGGACA TAATGATTA | |
| TGCCGACTIC | 540 |
| GATAAAAACC GGCGCAAGAGA | 540 |
| GATAAAAACC GGCGCAAGAG CTGCCAGGGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA TGTGGGTACC GCCTTGTGCG GAGACAGAGA | |
| TOTAL GTGGCTCCCG GAGAGAGAGA | 600 |
| ATGGTGAAGT GTGGCTCCCG GAGAGAGAGA. AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC AGTGCCGACG AGCAGCTGCA CTGTGCCGGC AAGGCCAAGA GAAGTGGCGG CCACGCGCCC | |
| AGGCTGCA CTGTGCCGGC AAGGCCCAA | 660 |
| AGTGCCGACG AGCAGCTGCA CTGTGCCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG CGAGTGCGGG AGCTGCTGCT GGACGCCCTG AGCCCCGAGC AGCTAGTGCT CACCCTCCTG | |
| AGCCCCTG AGCCCCGAGG | 720 |
| CGAGTGCGGG AGCTGCTGC1 GGAGGCCTCC | 720 |
| CGAGTGCGGG AGCTGCTGCT GGACGCCCAGTG CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC GAGGCTGAGC CGCCCCATGT GCTGATCAGC CGCCCCAGTG CGCCCTTCAC CGAGGCCTCC | |
| 15 CONTIGAGE COCCCCATGT GCTGATCAGG | 780 |
| GAGGCIGAGE CAGCIGAT CAGCIGAT | |
| CONTRACCAA GTTGGCCGAC AAGGATO | 840 |
| ATGATGATGT CCCTGACCAA GTTGGCCGAC AAGGAGTTGG TACACATGAT CAGCTGGGCC AAGGAGATTC CCCGGCTTTGT GGAGCTCAGC CTGTTCGACC AAGTGCGGCT CTTGGAGAGC AAGAAGATTC CCGGCTTTGT GGAGCTCAGC CTGTTCGACC CAATTGACCA CCCCGGCAAG | |
| 20 CTGTTCGCC CTGTTCGCCC | 900 |
| AAGAAGATTC CCGGCTTTG1 | 900 |
| ATGTGGGGG. | |
| CONCENTRAT GATGOGGG | 960 |
| 25 ACCCATGAGG GGAAATGCGI MARA | |
| TCCAGATCT TGTTCTGGAC AGGGT | 1020 |
| 25 TGTTGGATGG AGGTGTTAAT GATGGGGT CTCATCTTTG CTCCAGATCT TGTTCTGGAC AGGGATGAGG GGAAATGCGT AGAAGGAATT CTCATCTTTG CTCCAGATCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT AAAACTCCAA CTGGAAATCT TTGACATGCT CCTGGCAACT ACTTCAAGGT TTCGAGAGTT GTACCCTCT | • |
| ACTICAL ACTICANOS | |
| CTGGAAATCT TTGACATGCT CCTGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCT CACAAAGAAT ATCTCTGTGT CAAGGCCATG ATCCTGCTCA ATTCCAGTAT GTACCCTCT | G 1000 |
| COCCATG ATCCTGCT | |
| CACARAGAAT ATCTCTGTGT CAACGCCGT | rg 1140 |
| CACAAAGAAT ATCTCTGTGT CAAAAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGT GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGT GTCACAGCGA CCCAGGATGC TGACAGCAGC CGGAAGCTGG CTCACTTGCT GAACGCCGT | |
| 35 COCAGGATGC TGACAGCAGC COCAGGATGC | TG 1200 |
| GTCACAGCA GCAATCC | |
| GTCACAGCGA CCCAGGATGC TGACAGCAG AGCGGCATCT CCTCCCAGCA GCAATCCA ACCGATGCTT TGGTTTGGGT GATTGCCAAG AGCGGCATCT CCTCCCAGCA GCAATCCA | 1257 |
| ACCGATGCTT TGGTT1GGT | 120 |
| CTCCTGTCC CACGTCAGGC AT GOOD | |
| ACCGATGCTT TGGTTTGGGT GATTOTTCC CACGTCAGGC ATGCGAGGTC TGCCTGA 40 CGCCTGGCTA ACCTCCTGAT GCTCCTGTCC CACGTCAGGC ATGCGAGGTC TGCCTGA | |
| 21: | |
| (2) INFORMATION FOR SEQ ID NO: 21: | |
| (2) INFORMATION | |
| (i) SEQUENCE CHARACTERISTICS: | • |
| (i) SEQUENCE CHARGE And acids (A) LENGTH: 418 amino acids | |
| (A) LENGTH (A) (B) TYPE: amino acid | |
| (B) TYPE: americs: single | |
| (C) STRANDEDNESS: single | |
| (D) TOPOLOGY: linear | |
| | |
| 55 (ii) MOLECULE TYPE: protein | |
| 55 (ii) MOLECCE | |

| | | (xi) SEQUENCE DESCRI | SEO ID NO: 21 | l: | |
|------------------------|----|-----------------------|----------------------------|------------------|---------------------|
| and the control of the | 5 | SEQUENCE DESCRIP | PTION: SLE | _ | al. Pro |
| | 5 | (xi) SEQUENCE DESCRIP | 1 Th | r Asn Leu Glu Gl | TA GTA LIO |
| . • | | ser Ile | Pro Ser Ash Val | | 15 |
| | | Met Asn Tyr Ser 5 | 10 | • | |
| • | | 1 | | Thr P | ro Gly His |
| | 10 | Gly Arg Gln Thr Th | a pro Asn Val Le | eu Trp Pro 1111 | 20 |
| | | Slu Ard Gln Thr Th | r Ser Flo | • | ,0 |
| | | 20 | | | (1) |
| | | | al Val His Arg Gln 1 | Ten Ser His Leu | Tyr Ala Gru |
| | 15 | - ten V | al Val His Arg Gin ' | 45 | |
| | | Leu Ser Pro Dea | 40 | | |
| | | 35 | | Clu | His Thr Leu |
| | | | - cua Glu Ala | Arg Ser Leu Glu | |
| • | 20 | pro Gln Lys Ser F | ero Trp Cys Glu Ala 55 | 60 | |
| | J. | 50 | 50 | | Sum CVS |
| | | Ju | | Tus Val Ser Gl | Asn Arg Cir |
| | | Ard | Glu Thr Lou Lys Arg | 75 | 80 |
| | 25 | Pro Val Ash Any | 70 | 13 | |
| * * | 25 | 65 | Thr Gly Pro Gly Se | 3 m A1 | a His Phe Cys |
| | | | Cly Pro Gly Se | er Lys Arg Asp A | 95 |
| • | | ala Ser Pro Val | The Gry | 90 | • |
| | | Are | 85 | | Set |
| | 30 | | r Asp Tyr Ala Ser G | TVE HIS TYP | ily Val Trp 505 |
| | | . com Se | r Asp Tyr Ala Ser G | ità •1- | 110 |
| • | | Ala Val Cys 10 | - .0 | [05 | |
| • | | 10 | ,0 | | Gin Gly His Asn |
| | 35 | | ys Lys Ala Phe Phe | Lys Arg Ser 11e | 125 |
| • | | Cys Glu Gly C | ys Lys AL 120 | | 124 |
| | | 115 | | | Tan Arg |
| · | | | Cys Pro Ala Thr Asn 135 | Gln Cys Thr Ile | Asp Lys Asi |
| | 40 | m 71# (| Cys Pro Ala Thr Ash | 140 |) |
| | | Asp Tyr 110 | 135 | | _ |
| • | | 130 | Cys Gln Ala Cys Ar | CV | a Tyr Glu Val Gly |
| | | | - cln Ala Cys Ar | g Leu Arg Lys 01 | 160 |
| | 45 | Arg Lys Ser | 150 | 155 | • |
| | | 145 | 130 | | ard Leu Val |
| | | *** | 3 Cys Gly Ser Arg A | ra Glu Arg Cys G | ly Tyr my 175 |
| | | Val LVS | Cys Gly Ser Arg A | 170 | 113 |
| | 50 | Met var -1 | 165 | - | . • • · |
| | 50 | | | uin | Cys Ala Gly Lys Ala |
| | | | ser Ala Asp | Glu Gln Leu His | Cys Ala Gly Lys Ala |
| · | • | Arg Arg Gl | n Arg 502 | | |
| <u> </u> | | | | | |
| 4 | 55 | | | | |

| | | | 190 | |
|----|------------------------------------|-------------------|------------------|-------------|
| | 180 | 185 | | |
| | 180 | | Tau | Ano |
| | Lys Arg Ser Gly Gly His Ala | Pro Arg Val Arg | Slu Leu Leu Leu | 1-F |
| | Lug Arg Ser Gly Gly His Ala | -00 | 205 | |
| 5 | 105 | | | |
| | Ala Leu Ser Pro Glu Gln Leu 215 | | Glu Ala Glu | 1 Pro |
| | can Gin Leu | Val Leu Thr Leu | Den Gra | |
| | Ala Leu Ser Pro Giu 511 215 | | 220 | |
| | | | | |
| 10 | 210 Pro His Val Leu Ile Ser Arc | 31: Pro | Phe Thr Glu Al | a Ser |
| | I Leu Ile Ser Arg | pro Ser Ala 125 | | 240 |
| | Pro His val 230 | 235 | | |
| | | | 1 W | ie Wet |
| 15 | 225 Met Met Met Ser Leu Thr Ly | Tau Ala Asp Lys | Glu Leu Val A. | |
| | Mor Met Met Ser Leu Thr Ly | 250 | 2 | 55 |
| | | | | |
| | Ile Ser Trp Ala Lys Lys I | | . cl., Leu Ser I | eu Phe |
| | TUE LUE I | le Pro Gly Phe Va | 7 GIR 150 - | |
| 20 | Ile Ser Trp Ala Lys Bit | 265 | 210 | |
| | | | | |
| | Asp Gla Val Arg Leu Leu | our TED N | et Glu Val Leu | Net Met |
| | ot Val Ard Lou Lou | alu Ser Cys IIP | 285 | |
| 25 | Asp GLE VEZ | 280 | _ | |
| | 275 | | - 710 | Dhe Ala |
| | Gly Leu Met Trp Arg Ser | TIA BED His Pro G | ly Lys Leu 116 | the |
| | Gly Leu Met Trp Arg Ser | ITG 1mb | 300 | |
| | | _ | | |
| 30 | 250 | | Val Gli | a Gly Ile |
| | 290 Pro Asp Leu Val Leu Asp | Arg Asp Glu Gly | LAR CAR 1- | 320 |
| | Pro Asp Leu Vai neu 310 | | 315 | |
| | 3 | | | |
| 35 | Leu Glu Ile Phe Asp Me | ale Thr | The Ser Arg Ph | ie Arg Giu |
| | Clastle Phe Asp Me | t Leu Leu Ala 102 | | 335 |
| | Leu Gru 125 | 330 | 1 | |
| | 55 | | -1 - M | or the Leu |
| | Leu Lys Leu Gln His L | Clu TVI Leu Cys | 5 Val Lys Ala M | |
| 40 | Leu Lys Leu Gln His L | 78 524 -1- | 3 | 150 |
| | | | | |
| | Leu Asn Ser Ser Met I | | ala The Gla | Asp Ala Asp |
| | - cor Met T | yr Pro Leu Val Th | 365 | |
| 45 | Leu Asn Ser Ser 1100 | 360 | 303 | : |
| | | | | |
| | | Ten A | sn Ala Val Thr | Asp Ala Leu |
| | Ser Ser Arg Lys Leu | Ala His Leu Leu A | 380 | |
| | | | | |
| 50 | 370 | | | Cin Ser Met |
| | 370 Val Trp Val Ile Ala | Ser Gly Ile | Ser Ser Gln Gln | 1 544 - 5- |
| , | Val Trp Val Ile Ala | DAS DOX 223 | | |
| • | • | | | |
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| | 395 390 | |
|----|---|----|
| | on a profite Ala Arg | |
| 5 | Arg Leu Ala Asn Leu Leu Met Leu Leu Ser His Val Arg His Ala Arg 415 405 | |
| 10 | Ser Ala | |
| | (2) INFORMATION FOR SEQ ID NO: 22: | |
| 15 | (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 base pairs | |
| 20 | (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear | |
| 25 | (ii) MOLECULE TYPE: cDNA | |
| 30 | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22: | |
| | CTTGGATCCA TAGCCCTGCT GTGATGAATT ACAG | • |
| 35 | (2) INFORMATION FOR SEQ ID NO: 23: | |
| | (i) SEQUENCE CHARACTERISTICS: | |
| | (A) LENGTH: 33 base pairs | |
| 40 | (B) TYPE: nucleic acid (C) STRANDEDNESS: single | |
| | (C) STRANDEDESS: CONTROL (D) TOPOLOGY: linear | |
| | (D) TOPOLOGY: 2-11 | |
| 45 | (11) MOLECULE TYPE: cDNA | |
| 50 | | 33 |
| | GATGGATCCT CACCTCAGGG CCAGGCGTCA CTG | |

(2) INFORMATION FOR SEQ ID NO: 24:

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(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 1898 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CACGAATCTT TGAGAACATT ATAATGACCT TTGTGCCTCT TCTTGCAAGG TGTTTTCTCA 60 SCTSTTATCT CAAGACATGG ATATAAAAAA CTCACCATCT ASCCTTAATT CTCCTTCCTC 120 CTACAACTGC AGTCAATCCA TCTTACCCCT GGAGCACGGC TCCATATACA TACCTTCCTC 180 CTATGTAGAC AGCCACCATG AATATCCAGC CATGACATTC TATAGCCCTG CTGTGATGAA 240 TTACAGCATT CCCAGCAATG TCACTAACTT GGAAGGTGGG CCTGGTCGGC AGACCACAAG 300 CCCAAATGTG TTGTGGCCAA CACCTGGGCA CCTTTCTCCT TTAGTGGTCC ATCGCCAGTT 360 ATCACATCTG TATGCGGAAC CTCAAAAGAG TCCCTGGTGT GAAGCAAGAT CGCTAGAACA 420 CACCTTACCT GTAAACAGAG AGACACTGAA AAGGAAGGTT AGTGGGAACC GTTGCGCCAG 480 CCCTGTTACT GGTCCAGGTT CAAAGAGGGA TGCTCACTTC TGCGCTGTCT GCAGCGATTA 540 600 CGCATCGGGA TATCACTATG GAGTCTGGTC GTGTGAAGGA TGTAAGGCCT TTTTTAAAAG AAGCATTCAA GGACATAATG ATTATATTTG TCCAGCTACA AATCAGTGTA CAATCGATAA 660 AAACCGGCGC AAGAGCTGCC AGGCCTGCCG ACTTCGGAAG TGTTACGAAG TGGGAATGGT 720

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| GAAGTGTGGC | TCCCGGAGAG | AGAGA10100 | GIACCGCCII | 010coananc | AGAGAAG16C | , 00 |
|-------------------|------------|------------|------------|------------|-------------|------------|
| CGACGAGCAG | CTGCACTGTG | CCGGCAAGGC | CAAGAGAAGT | GGCGGCCACG | CGCCCCGAGT | 840 |
| GCGGGAGCTG | CTGCTGGACG | CCCTGAGCCC | CGAGCAGCTA | GTGCTCACCC | TCCTGGAGGC | 900 |
| TGAGCCGCCC | CATGTGCTGA | TCAGCCGCCC | CAGTGCGCCC | TTCACCGAGG | CCTCCATGAT | 960 |
| GATGTCCCTG | ACCAAGTTGG | CCGACAAGGA | GTTGGTACAC | ATGATCAGCT | GGGCCAAGAA | 1020 |
| GATTCCCGGC | TTTGTGGAGC | TCAGCCTGTT | CGACCAAGTG | CGGCTCTTGG | AGAGCTGTTG | 1080 |
| GATGGAGGTG | TTAATGATGG | GGCTGATGTG | GCGCTCAATT | GACCACCCCG | GCAAGCTCAT | 1140 |
| CTTTGCTCCA | GATCTTGTTC | TGGACAGGGA | TGAGGGGAAA | TGCGTAGAAG | GAATTCTGGA | 1200 |
| AATCTTTGAC | ATGCTCCTGG | CAACTACTTC | AAGGTTTCGA | GAGTTAAAAC | TCCAACACAA | 1260 |
| AGAATATCTC | TGTGTCAAGG | CCATGATCCT | GCTCAATTCC | AGTATGTACC | CTCTGGTCAC | 1320 |
| AGCGACCCAG | GATGCTGACA | GCAGCCGGAA | GCTGGCTCAC | TTGCTGAACG | CCGTGACCGA | 1380 |
| TGCTTTGGTT | TGGGTGATTG | CCAAGAGCGG | CATCTCCTCC | CAGCAGCAAT | CCATGCGCCT | 1440 |
| GGCTAACCTC | CTGATGCTCC | TGTCCCACGT | CAGGCATGCG | AGTAACAAGG | GCATGGAACA | 1500 |
| TCTGCTCAAC | ATGAAGTGCA | AAAATGTGGT | CCCAGTGTAT | GACCTGCTGC | TGGAGATGCT | 1560 |
| GAATGCCCAC | GTGCTTCGCG | GGTGCAAGTC | CTCCATCACG | GGGTCCGAGT | GCAGCCCGGC | 1620 |
| AGAGGACAGT | AAAAGCAAAG | AGGGCTCCCA | GAACCCACAG | TCTCAGTGAC | GCCTGGCCCT | 1680 |
| GAGGTGAACT | GGCCCACAGA | GGTCACAAGC | TGAAGCGTGA | ACTCCAGTGT | GTCAGGAGCC | 1740 \$ |
| TGGGCTTCAT | CTTTCTGCTG | TGTGGTCCCT | CATTTGGTGA | TGGCAGGCTT | GGTCATGTAC | 1800 |
| 01 m c 000m c c c | | CAACTCTCAC | CACTCCCTCT | GAGGAAGCCA | TACTUME COM | 1860 |

TGTTAGCAGA GGGACATTTG AATCGAGCGT TTCCACAC

(2) INFORMATION FOR SEQ ID NO: 25:

| 3 | | | | | | | | | | | | | | | |
|----|---------|--------------|-------|-----------|-------|----------|------|-----|-------|-------------|-----|-----|-----------|-------|-----|
| | (i) SE | QUENCE | CHAI | RACTI | ERIS1 | rics | : | | | | | | | | |
| | (| A) LEN | GTH: | 530 | ami | no a | cids | | | | | | | | |
| 10 | | B) TYP | | | | | | | | | | | | | |
| | | (C) STR | | | | | e | | | | | | | | |
| | (| (D) TOP | OLOG' | Y: 1: | inea | <u> </u> | | | | | | | | | |
| 15 | (ii) MC | LECULE | TYP | E: p | eptio | de | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | | | |
| | (xi) SI | equence | DES | CRIP | TION | : SE | Q ID | NO: | 25: | | | | | | |
| 25 | Met A | sp Ile | Lvs | Asn | Ser | Pro | Ser | Ser | Leu | As n | Ser | Pro | Ser | Ser | Tyr |
| | 1 | - - - | | 5 | | | | | 10 | | | | | 15 | |
| | | | | | | | | | | | | | | _ | |
| 30 | Asn C | ys Ser | | Ser | Ile | Leu | Pro | | Glu | His | Gly | Ser | Ile 30 | Tyr | Ile |
| 30 | | | 20 | | | | | 25 | | | | | 30 | | |
| | Dro 9 | er Ser | TVI | Val | Asp | Ser | His | His | Glu | Tyr | Pro | Ala | Met | Thr | Phe |
| | | 35 | -3- | | • | | 40 | | | | | 45 | | | |
| 35 | | | | | | | | | | | | | | | |
| | Tyr S | er Pro | Ala | Val | Met | | Tyr | Ser | Ile | Pro | | Asn | Val | Thr | Asn |
| | 5 | 0 | | | | 55 | | | | | 60 | | | | |
| 40 | | ilu Gly | G1 | Dwa | C1 | Ara | Gl n | Thr | Thr | Ser | Pro | Asn | Val | Leu | Trp |
| | Leu G | era era | GIÅ | FLO | 70 | ,9 | · | | | 75 | | | | | 80 |
| | | | | | | | | | | | | | | | |
| 45 | Pro 7 | Thr Pro | Gly | His | Leu | Ser | Pro | Leu | Val | Val | His | Arg | Gln | | Ser |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| | | Leu Ty: | | 61 | . D | - C1- | T.ve | Set | Pro | Tro | Cvs | Glu | Ala | Arq | Ser |
| 50 | His 1 | Leu Tyr | 100 | | PIO | GII | гра | 105 | | | -,- | | 110 | | |
| | | | 100 | • | | | | | | | | | | | |
| | Leu | Glu His | s Thi | Lev | Pro | Va] | LAsr | Arç | , Glu | Thr | Leu | Ly: | a Arg | , Lys | val |
| 55 | | | | | | | | | | | | | | | |

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| 5 | Ser | Gly 130 | As n | Arg | Cys . | | Ser 135 | Pro | Val | Thr | | Pro 140 | Gly | Ser | Lys . | Arg |
|----|--------------------|-------------|-------------|-------------------|------------|-------------|------------|-------------------|------------|------------|------------|------------|------------|------------|-------------------|--------------|
| 10 | As p 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| | Tyr | Gly | Val | Тгр | Ser 165 | Cys | Glu | Gly | Сув | Lys 170 | Ala | Phe | Phe | | Arg 175 | Ser |
| 15 | Ile | Gln | Gly | His 180 | Asn | As p | Tyr | Ile | Cys 185 | Pro | Ala | Thr | Asn | Gln 190 | Суз | Thr |
| 20 | Ile | Asp | Lys 195 | Asn | Arg | Arg | Lys | Ser 200 | Сув | Gln | Ala | Cys | Arg 205 | Leu | Arg | Lys |
| 25 | Cys | Tyr 210 | | Val | Gly | Met | Val 215 | Lys | Cys | Gly | Ser | Arg 220 | Arg | Glu | Arg | Cys |
| 30 | Gly 225 | | Arg | Leu | Val | Arg 230 | Arg | Gln | Arg | Ser | Ala 235 | Авр | Glu | Gln | Leu | His 240 |
| | Cys | Ala | Gly | Lys | Ala 245 | | Arg | Ser | Gly | Gly 250 | | Ala | Pro | Arg | Val 255 | Arg |
| 35 | Glu | Lev | Leu | Leu 260 | | Ala | Leu | Ser | 265 | | Gln | Leu | Val | Leu 270 | | Leu |
| 40 | Let | ı Glı | 275 | | Pro | Pro | Hi: | Val 280 | | ı Ile | : Ser | : Arg | 285 | | : Ala | Pro |
| 45 | Pho | e Th. 29 | | u Ala | a Sei | r Met | 29: | | t Se. | r Leı | 1 Thi | 30° | | ı Ala | a As _i | Lys |
| 50 | G1 30 | | u Va. | l Hi | s Me | t Il | | r Tr | p Al | a Ly | s Ly: | | e Pr | Gly | y Ph | e Val 320 |
| | G1 | u Le | u Se | r Le | u Ph | e As | p Gl | n Va | l Ar | g Le | u Le | u Gl | u Se | r Cy | s Tr | p Met |

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| 5 | Glu | Val | Leu | Met 340 | Met | Gly | Leu | Met | Trp 345 | Arg | Ser | Ile | Азр | His 350 | Pro | Gly |
|----|------------|--------------------|-------------------|-------------------|------------|------------|------------|------------|------------|------------|-------------------|-------------------|--------------------|------------|------------|------------|
| 10 | Lys | Leu | 11e 355 | Phe | Ala | Pro | Asp | Leu 360 | Val | Leu | Asp | Arg | As p 365 | Glu | Gly | Lys |
| 15 | Cys | Val 370 | Glu | Gly | Ile | Leu | Glu 375 | Ile | Phe | Asp | Met | Leu 380 | Leu | Ala | Thr | Thr |
| | Ser 385 | Arg | Phe | Arg | Glu | Leu 390 | Lys | Leu | Gln | His | Lys 395 | Glu | Tyr | Leu | Cys | Val 400 |
| 20 | Lys | Ala | Met | Ile | Leu 405 | Leu | Asn | Ser | Ser | Met 410 | Tyr | Pro | Leu | Val | Thr 415 | Ala |
| 25 | Thr | Gln | Asp | Ala 420 | Авр | Ser | Ser | Arg | Lys 425 | Leu | Ala | His | Leu | Leu 430 | Asn | Ala |
| 30 | Val | Thr | Asp 435 | Ala | Leu | Val | Trp | Val 440 | Ile | Ala | Lys | Ser | Gly 445 | Ile | Ser | Ser |
| 35 | Gln | Gln 45 0 | Gln | Ser | Met | Arg | Leu 455 | Ala | Asn | Leu | Leu | Met 460 | Leu | Leu | Ser | His |
| | Val 465 | Arg | His | Ala | Ser | Asn 470 | Lys | Gly | Met | Glu | H1s 475 | Leu | Leu | Asn | Met | Lys 480 |
| 40 | Суз | Lys | Asn | Val | Val 485 | Pro | Val | Туг | Asp | Leu 490 | Leu | Leu | Glu | Met | Leu 495 | Asn |
| 45 | Ala | His | Val | Leu 500 | Arg | Gly | Суз | Lys | Ser 505 | | Ile | Thr | Gly | Ser 510 | Glu | Суз |
| 50 | Ser | Pro | Ala 515 | | Asp | Ser | Lys | Ser 520 | | Glu | Gly | Ser | Gln 525 | | Pro | Gln |
| | Ser | Gln | | | | | | | | | | | | | | |

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(2) INFORMATION FOR SEQ ID NO: 26:

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| | (i) SEQUENCE CHARACTERISTICS: | |
|----|---|----|
| | (A) LENGTH: 30 base pairs | |
| 10 | (B) TYPE: nucleic acid | |
| | (C) STRANDEDNESS: single | |
| | (D) TOPOLOGY: linear | |
| 15 | (ii) MOLECULE TYPE: other nucleic acid | |
| 20 | | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26: | |
| 25 | GTGCGGATCC TCTCAAGACA TGGATATAAA | 30 |
| | (2) INFORMATION FOR SEQ ID NO: 27: | |
| 30 | | |
| | (1) SEQUENCE CHARACTERISTICS: | |
| | (A) LENGTH: 25 base pairs | |
| 35 | (B) TYPE: nucleic acid | |
| | (C) STRANDEDNESS: single | |
| | (D) TOPOLOGY: linear | |
| 40 | (ii) MOLECULE TYPE: other nucleic acid | |
| 45 | | |
| | (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27: | |
| 50 | AGTAACAGGG CTGGCGCAAC GGTTC | 25 |
| | (2) INFORMATION FOR SEQ ID NO: 28: | |
| | (i) SPOUPNCE CHARACTERISTICS: | |

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

ACTGGCGATG GACCACTAAA GG

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25 Claims

- Isolated DNA encoding a protein having an N-terminal domain, a DNA-binding domain and a ligand-binding domain, wherein the amino acid sequence of said DNA-binding domain of said protein exhibits at least 80% homology with the amino acid sequence shown in SEQ ID NO:3, and the amino acid sequence of said ligand-binding domain of said protein exhibits at least 70% homology with the amino acid sequence shown in SEQ ID NO:4.
- Isolated DNA according to claims 1, characterized in that the amino acid sequence of said DNA-binding domain
 of said protein exhibits at least 90%, preferably 95%, more preferably 98%, most preferably 100% homology with
 the amino acid sequence shown in SEQ ID NO:3.

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- Isolated DNA according to claims 1 or 2, characterized in that the amino acid sequence of said ligand-binding domain of said protein exhibits at least 75%, preferably 80%, more preferably 90%, most preferably 100% homology with the amino acid sequence shown in SEQ ID NO:4.
- Isolated DNA according to claims 1 to 3, said DNA encoding a protein comprising the amino acid sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.
 - Isolated DNA according to claims 1 to 4, characterized in that said DNA comprises the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:20 or SEQ ID NO:24.

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- 6. A recombinant expression vector comprising the DNA according to any of the claims 1 to 5.
- 7. A cell transfected with DNA according to claims 1 to 5 or an expression vector according to claim 6.
- 8. A cell according to claim 7 which is a stable transfected cell line which expresses the steroid receptor protein according to any of the claims 9 to 11.
 - 9. Protein encoded by DNA according to claims 1 to 5 or an expression vector according to claim 6.
- 10. Protein according to claim 9, said protein comprising the amino acid sequence of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:21 or SEQ ID NO:25.
 - 11. Chimeric protein having an N-terminal domain, a DNA-binding domain, and a ligand-binding domain,

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characterized in that at least one of said domains of said chimeric protein originates from a protein according to claims 9 or 10, and at least one of the other domains of said chimeric protein originates from another receptor protein from the nuclear receptor superfamily, provided that the DNA-binding domain and the ligand-binding domain of said chimeric protein originates from different proteins.

12. DNA encoding a protein according to claim 11.

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- 13. Use of a DNA according to claims 1 to 5 or 12, an expression vector according to claim 6, a cell according to claim 7 or 8 or a protein according to claim 9 to 11 in a screening assay for identification of new drugs.
- 14. A method for identifying functional ligands for the protein according to claims 9 to 11, said method comprising the steps of
 - a) introducing into a suitable host cell 1) DNA according to claims 1 to 5 or 12, and 2) a suitable reporter gene functionally linked to an operative hormone response element, said HRE being able to be activated by the DNA-binding domain of the protein encoded by said DNA;
 - b) bringing the host cell from step a) into contact with potential ligands which will possibly bind to the ligandbinding domain of the protein encoded by said DNA from step a);
 - c) monitoring the expression of the protein encoded by said reporter gene of step a).

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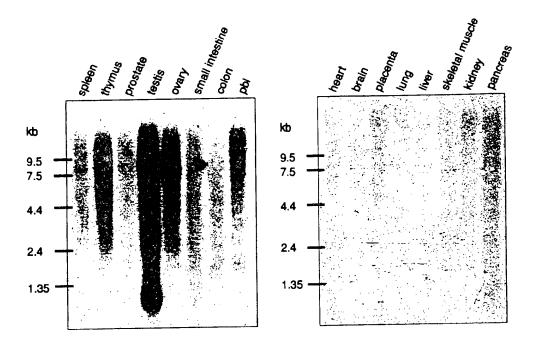
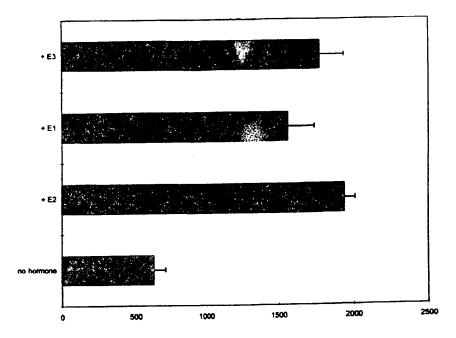


Figure 1

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luciferase units

Fig. 2

Transient transfection of CHO cells with Estrogen Receptors Alpha and Beta Incubation with Estradiol and ICI

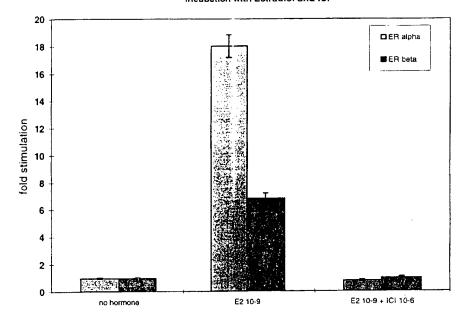


Figure 3

ERα and ERβ RT PCR on tissue-representative cell lines

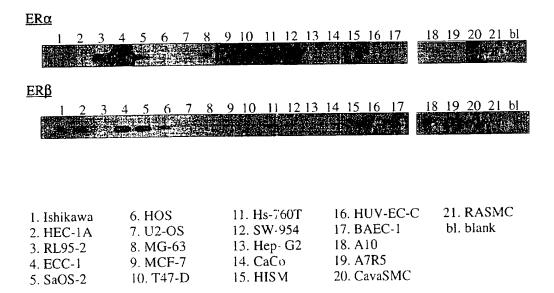


Figure 4

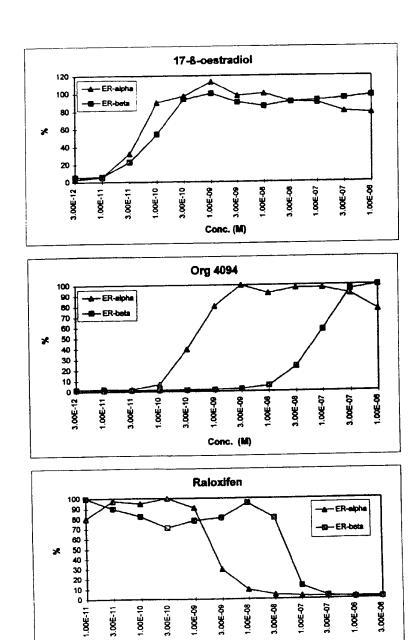


Figure 5

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(12)

EUROPEAN PATENT APPLICATION

- (88) Date of publication A3: 29.12.1997 Bulletin 1997/52
- (43) Date of publication A2: 01.10.1997 Bulletin 1997/40
- (21) Application number: 97200903.9
- (22) Date of filing: 25.03.1997

- (51) Int CL⁶. **C12N 15/12**, C12N 15/62, C12N 15/85, C07K 14/72, C12N 1/21, C12N 5/16, C12Q 1/00, C12Q 1/68
- (84) Designated Contracting States:
 AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
 NL PT SE
- (30) Priority: 22.11.1996 EP 96203284 26.03.1996 EP 96200820
- (71) Applicant: Akzo Nobel N.V. 6824 BM Arnhem (NL)
- (72) Inventors:
 Mosselman, Sietse
 5346 VM OSS (NL)

- Dijkema, Rein
 5345 ML Oss (NL)
- (74) Representative: Ogilvie-Emanuelson, Claudia Maria et al Patent Department Pharma N.V. Organon P.O. Box 20 5340 BH Oss (NL)

(54) Estrogen receptor

(57) The present invention relates to isolated DNA encoding novel estrogen receptors, the proteins encod-

ed by said DNA, chimeric receptors comprising parts of said novel receptors and uses thereof.



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EUROPEAN SEARCH REPORT

Application Number EP 97 20 0903

| ategory | Citation of document with inc | | Relevant | CLASSIFICATION OF THE APPLICATION (INLC).6) |
|----------------|--|--|--|--|
| (,D | human estrogen recep DNA." SCIENCE, vol. 231, 13 March 1 pages 1150-1154, XPG | ence and expression of otor complementary | 1-14 | C12N15/12 C12N15/62 C12N15/85 C07K14/72 C12N1/21 C12N5/16 C12Q1/00 C12Q1/68 |
| Y | orphan receptors spe steroid receptor sup PROCEEDINGS OF THE N SCIENCE, vol. 91, June 1994, pages 6040-6044, XPC * the whole document | NATIONAL ACADEMY OF | 1-14 | |
| A,D | EVANS, R.M.: "The hormone receptor support of the second o | 1-14 | TECHNICAL FIELDS SEARCHED (Int.CL6) C12N C07K C12Q | |
| A | EP 0 371 820 A (SAL STUDI) 6 June 1990 * page 4, lines 20- | K INST FOR BIOLOGICAL 27 and claim 19 * | 11-14 | |
| E | WO 97 09348 A (KARO J M (SE); ENMARK EV * the whole documen | | 3 1-14 | |
| | The present search report has b | peen drawn up for all claims | | Examples |
| | i | 23 October 1997 | Ma | ind1, B |
| X par Y par | THE HAGUE CATEGORY OF CITED DOCUMENTS Tricularly relevant if taken alone ticularly relevant if combined with anoth ument of the same category horiogoal background | T : theory or princp E : earlier patent do after the filing do | ole underlying the scument, but put ste in the applicatio for other reason | e invention clished on, or |

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EUROPEAN SEARCH REPORT

Application Number EP 97 20 0903

| | DOCUMENTS CONSIDE | | | CLASSIFICATION OF THE |
|--|--|---|---|--|
| Category | Citation of document with ind of relevant passa | | Rele to cla | |
| Y,D | human estrogen reception." SCIENCE, vol. 231, 13 March pages 1150-1154, XPC | 1986. | | C12N15/12 C12N15/62 C12N15/85 C07K14/72 C12N1/21 C12N5/16 C12Q1/00 C12Q1/68 |
| Y | orphan receptors spi steroid receptor su PROCEEDINGS OF THE SCIENCE, vol. 91, June 1994, pages 6040-6044, XP * the whole documen | NATIONAL ACADEMY OF | 1-14 | |
| A,D | EVANS, R.M.: "The hormone receptor su SCIENCE, vol. 240, 13 May 19 pages 889-895, XP00 * the whole documen | perfamily." 88, 2019515 | 1-14 | TECHNICAL FIELDS SEARCHED (Int.CL6) C12N C07K C12Q |
| Α | EP 0 371 820 A (SAL STUDI) 6 June 1990 * page 4, lines 20- | K INST FOR BIOLOGICAL 27 and claim 19 * | 11-1 | .4 |
| Ε | WO 97 09348 A (KARO J M (SE); ENMARK EV * the whole documen | | G 1-14 | 1 |
| | The present search report has | been drawn up for all claims | | |
| | Place of search | Date of completion of the search | · | Examiner |
| | THE HAGUE | 23 October 199 | 7 | Mandl, B |
| X pa. Y:pai doc A:ted O:no | CATEGORY OF CITED DOCUMENTS riscularly relevant if taken alone riscularly relevant if combined with anot purpose to the same partegory thrological background in-written disclosure ermediate document | E : earlier patent after the filling her D : document oft L : document oft | t document, i date led in the app ed for other r | reasons |



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EUROPEAN SEARCH REPORT

Application Number EP 97 20 0903

| | DOCUMENTS CONSIDERE Citation of document with indicate | | Relevant | CLASSIFICATION OF THE |
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| ategory | of relevant passages | | to claim | APPLICATION (Int.Cl.6) |
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| | | | | TECHNICAL FIELDS SEARCHED (Int Cl.6) |
| | | | | |
| | The present search report has been | drawn up for all claims | | |
| | Place of search | Date of completion of the search | | Examiner |
| | THE HAGUE | 23 October 1997 | Má | andl, B |
| CATEGORY OF CITED DOCUMENTS X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O non-writen disologure | | | locument, but put tate d in the application of for other reason | blished on, or on |